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A NINE YEARS' FIELD EXPERIMENT ON THE IMMUNISATION OF CATTLE AGAINST TUBERCULOSIS.

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GENERAL INTRODUCTION.

THE experiments here described were carried out at Söllestedgaard Manor, Lolland, Denmark. The dairy herd on this estate was very heavily infected with tuberculosis, and the owner, Mr. Theophil Jörgensen, asked one of us (G. D.) if he would like to attempt to immunise the herd with Diaplyte vaccine (Dreyer, 1923). We were offered a free hand in planning and carrying out the experiment, but, naturally, undue interference with the commercial management of the herd had to be avoided.

The first part of the experiment was planned and carried out in collaboration with Professor Holger Möllgaard, M.D., of the Royal Veterinary High School, Copenhagen, who was interested in the therapeutic use of Sanocrysin, while we were chiefly interested in the prophylactic use of " diaplyte " vaccine. The tuberculin tests, Sanocrysin treatment, and the early injections of vaccine were carried out by Professor Möllgaard's assistant, Mr. O. Olsen, Veterinary Surgeon. Since the middle of 1925, all tuberculin tests, vaccine injections, and *post-mortem* examinations have been made by Mr. H. H. Ammitzböll, the veterinary surgeon to the estate.

The general plan of the experiment, the preparation of the vaccine, and the scheme of dosage of the vaccine was undertaken by the two of the authors in Oxford (G. D. and R. L. V.). One or both of the Oxford collaborators were in Denmark for a considerable time during each summer vacation, when the records were inspected and analysed, and further plans prepared.

History and Management of the Herd.

The herd consists of " red Danish " cows. Up till about 1885 there were approximately 160 milch cows and 80 heifers and calves. It was at this time free of tuberculosis, as judged by clinical observation, and was one of the better herds of dairy cattle in the island of Lolland. About 1885, a number of steers were bought for fattening, and these animals unfortunately introduced tuberculous infection into the herd. Tuberculosis gradually spread, and largely as a result of this infection the herd had been greatly reduced by 1924. By this time all the milch cows, and 73 per cent. of the bulls, heifers and calves were infected. We then, with considerable trepidation, undertook an attempt to immunise the uninfected cattle and the calves subsequently born.

Before going into details of the experiment some description of the conditions on the farm and of the management of the herd must be given. During the winter months, from about November 1st to May 1st, the cattle are kept in the stable. This is a comparatively modern building, with adequate window surface and ventilation. The floor is of concrete. The cattle are tied by stanchions which permit comfortable but not excessive movement. The cows are placed in rows, head to head, with a 4-ft. 7-in. gangway between the rows for the distribution of fodder. A common drinking trough runs along the gangway, and is shared by all cows in the row. The position of the cows in the stable is determined by the type of fodder they are given. The fodder ration depends on the yield and analysis of the milk. The cows are moved from one stall to another as the rations are varied, according to the results of the monthly analysis of the milk, and no attempt is made to separate the old animals from the young ones, nor the known tuberculous animals from the uninfected ones. Thus there is adequate opportunity for the spread of infection from one animal to another.

The calves in the stable are kept in a series of boxes or pens, where they are allowed to mix freely. They have a common drinking trough and a common supply of fodder. If any one happens to be infected, there is therefore ample opportunity to communicate the infection to its neighbours.

During the summer the cattle are out in the fields or meadows. The milch cows are tethered in a field of clover or lucerne and grass so that each one can graze over a circle about 20-ft. in diameter. The line of cows is moved forward at frequent intervals. Water is brought to the tethered animals, and all drink from the same vessels. The heifers and calves are allowed to graze freely in one of the three meadows, or in the field which has immediately before been used for the milch cows. No attempt is made to keep infected and non-infected animals in separate meadows.

Calves, whether born in the meadow during the summer or in the stable during the winter, usually suck the mother for a week.

They are then placed in the calf pens and are fed on the mother's raw milk, or on the mixed raw milk for two weeks. During the next two weeks they are given raw milk and pasteurised skim milk, returned from the creamery, and after they are five weeks old they are given no more raw milk but only pasteurised skim milk.

If the mother is obviously clinically tuberculous the calf is not allowed to suck, and the milk from such cows is not used to feed the calves. It is quite possible, however, that infected cows may excrete tubercle bacilli in the milk, even when there is no apparent tuberculous lesion of the udder, and before the disease in other organs has become clinically obvious. There is, therefore, a possibility of infection through milk during the first weeks of the calf's life. Infection may also be spread at this time by the staff, who take no precautions to avoid carrying infectious material from infected cows to the calves. Groups of calves are kept together in adjoining pens, and one infected animal may spread infection to the others.

It is thus apparent that from the time the calf is born it may be exposed to infection, and so long as it remains in the herd it has ample opportunity during winter and summer, and both before and after taking its place with the milch cows, of acquiring tuberculous infection both through feeding and by contact.

Identification of Animals.

The usual Danish system of numbering and identifying dairy cattle is somewhat cumbersome. Each new born calf is given the same number as its mother. This " ear " number is indicated by a series of from one to six clips in definite positions on the right or left ear, or both. These marks in the ear remain and are legible more or less indefinitely, unless the ear is accidentally torn. When the heifer becomes a cow it is given a new number—the cow number—which is branded on the horns. The cow numbers are not assigned in sequence. If a cow dies, or has been sold, its number is subsequently assigned to another animal. This practice may be a source of some confusion, as it is possible that during a period of ten years five or six animals may have had the same cow number, although, of course, only one animal would have the number at any one time. Therefore when referring to animals which have been in the herd some years previously, it is necessary to know both the date of birth and the cow number in order to identify the animal. To obviate this difficulty, for purposes of the experiment each animal was given a serial number called the Experimental Number. These experimental numbers were stamped on aluminium discs which are readily fixed through a small hole punched in the ear. On the rare occasions when one of these aluminium discs was accidentally torn off, the animal could be identified by its ear number, or cow number, and the disc replaced. The disc, stamped with the experimental number

is attached to the ear immediately after the calf is born. This system has been found to work very satisfactorily.

Plan of Experiment.

In an experiment on the immunisation of a herd of cattle against tuberculosis it would naturally be desirable to keep the herd as an isolated unit, and to follow each animal from the time of its birth to an eventual *post-mortem* examination. In a practical field experiment which must be conducted without undue interference with the commercial management of the herd, such a course is obviously impossible.

The original plan was first to test each animal in the herd with tuberculin. Half the non-reactors were to be immunised, leaving the other half as controls. Of the calves subsequently born, two-thirds were to be immunised and one-third were to be left as controls. The vaccine to be used throughout the experiment was " Diaplyte vaccine " (Dreyer, 1923) consisting of killed bacilli from which a large proportion of the fats had been extracted. No living vaccine was used. The tuberculin positive animals in the herd provided a natural source of infection for the immunised and control animals. All experimental animals were to be tuberculin tested twice a year. The veterinary surgeon to the estate was to observe the clinical condition of the animals throughout the course of the experiment, and to conduct a *post-mortem* examination on all animals which died or were slaughtered on the estate.

Some modifications of this original plan were made by design—and some by force of circumstances. The most important change concerns the number of controls. Some time after the experiment started it became apparent to the owner of the herd that the vaccinated animals were in better condition than the controls, as judged by the results of the tuberculin test and by clinical examination. At his request the number of controls was reduced, and eventually all calves were vaccinated. Two of the animals which had been left as controls were treated after they had developed a positive tuberculin reaction.

Epidemics of foot-and-mouth disease which occurred from time to time interfered to some extent with the routine inoculations and tuberculin tests, which in some cases had to be omitted to avoid the danger of spreading the infection—and to comply with the quarantine regulations.

The clinical examination of the herd has been regularly carried out. *Post-mortem* examinations have been made of practically all animals which were sold to the local butcher, or were slaughtered for use on the estate. In the case of animals shipped to some more distant abattoir, although full *post-mortem* details are lacking, a complaint was received if any animal proved to be tuberculous.

The Composition of the Herd.

At the beginning of the experiment, September 30th, 1924, there were 169 animals in the herd. Of these, 105 were milch cows, all of which were positive to the tuberculin reaction. The heifers, calves and bulls numbered in all 64, of which 47 were tuberculin positive, and 17 tuberculin negative. Ten of the tuberculin positive animals, and the 17 tuberculin negative animals were selected as the nucleus of the experimental group, and were given experimental numbers. Of the ten tuberculin positive animals, five were treated with sanocrysin only; three were treated with sanocrysin and were subsequently vaccinated; two were vaccinated in the hope of preventing the spread of the tuberculous lesions. Of the 17 which were negative to the tuberculin test, nine were kept as controls, and eight were vaccinated. (*See* Table 1.)

It is of interest that of the 17 animals which were negative to the tuberculin test, the oldest was 13 months; one was twelve months; one was eleven months; two were ten months; one was nine months; one was seven months; one was six months; three were two months and six were one month or less. Out of the total of 169 animals in the herd, 140 were twelve months old or more, and of these only two were tuberculin negative. One hundred and fifty-nine of the 169 animals were six months or more old, and of these only eight were tuberculin negative.

It is thus apparent that at the beginning of the experiment a very high percentage of the herd was infected with tuberculosis, and that the chance of any calf remaining free from infection for more than one year was comparatively small.

The composition of the herd since the beginning of the experiment has naturally been constantly changing. Many animals have been sold on account of sterility or abortion, or because they were poor milk producers. Some have died of pneumonia, enteritis, or other disease. Several have been slaughtered for use on the estate—including a few slaughtered for experimental purposes. During the period of the experiment 43 animals of ages varying from five to 40 months have been purchased. Some of these reacted positively to the tuberculin test when they came into the herd. Thirty of these animals have been vaccinated. (*See* Table 5.) As the history of these animals before coming into the herd is not definitely known, and as all of them were several months old when first vaccinated, they have been treated as a separate group. The other 13 animals bought in were not vaccinated, and are considered together with the non-experimental animals in the herd.

The numbers of animals of various groups in the herd at the times of the tuberculin tests is shown in Table 2. Each of these groups is considered in detail in the following sections.

There are also 16 animals to which experimental numbers were assigned which do not appear in any of the groups shown in

Table 2. These received one or more doses of vaccine, but none of them were in the herd at the time of any of the regular tuberculin tests, and their tuberculin reactions were never determined. All were young calves, from one week to three months old at the time they went out of the herd. Some were killed—some died of enteritis, and some of pleuro-pneumonia. None of them showed any clinical symptoms of tuberculosis and no tuberculous lesions were found in any which were killed. They have not been included in the Vaccine Group as they were in the herd for only a very short time, and they were never tested with tuberculin.

Vaccine.

The vaccine employed throughout the experiment is essentially the " diaplyte " vaccine described by one of us (Dreyer, 1923), but some modifications have been introduced in the technique of preparation.

The culture used for the preparation of vaccine has been an old human strain, TB.100. Various media have been used for growing the organism—glycerine bouillon, Hartley's broth, and Petroff's synthetic medium. The strain TB.100 grows profusely on all these media. The age of the culture has varied from three to six weeks. The growth is filtered off through muslin, or filter paper, and the organisms washed with water once or twice to free them from the greater part of the medium. The growth is then transferred to formalin (commercial), and stored in this until wanted—from one to twelve months.

To prepare the vaccine, the organisms are transferred to fresh commercial formalin, to which has been added 1 per cent. of formic acid (about 250 grams TB—weighed wet—to 1 litre of solution). This mixture is heated in a water bath at 60-65° C. for four to six hours. The organisms are then filtered off through paper—washed with acetone, and transferred to Soxhlet thimbles. They are extracted in a Soxhlet apparatus with acetone, for two to four days. The extraction is facilitated if the solvent surrounding the thimble is kept near its boiling point by placing the apparatus deeper in the water bath—or by wrapping a duster about it to insulate it partially. The acetone in the flask is changed at intervals, and the extraction continued as long as any appreciable amount of extractive is obtained. The organisms at this time are usually still acid-fast, particularly in the larger clumps. To break up these clumps, the bacteria are ground up in a glass mortar with fresh formalin containing 1 per cent. formic acid, until a homogeneous suspension is obtained. This is then diluted with more acidified formalin, and the mixture again heated for four to six hours at 60-65° C. The organisms are then filtered off through paper. Filtration at this stage is usually very slow, as the filter is quickly clogged. The suspension may be allowed to sediment for a few hours and the supernatant formalin poured

off. The remainder is allowed to filter overnight. The residue is then washed with acetone, transferred to the Soxhlet apparatus, and again extracted for two to four days with acetone.

After this time the organisms are usually almost entirely non-acid-fast. If there is still any appreciable proportion of acid-fast elements, the process of heating in formalin plus formic acid and extraction with acetone is repeated a third time.

The extracted bacteria are dried in a sterile glass weighing bottle, and weighed dry. They are then suspended in sterile saline, and the suspension is diluted to contain 5 milligrams per c.c. Formalin is added to the suspension at the rate of 1 part in 2,000. The suspension may be prepared—using aseptic precautions—by grinding in a glass or agate mortar. This, however, is somewhat tedious when large quantities of vaccine have to be prepared, and we have found it more convenient to use a ball mill. The mill consists of a very heavy Pyrex jar, of about 5 litres capacity, with $\frac{1}{2}$-in. balls of stainless steel. The dried bacilli, weighed, are put into the mill, and enough saline added to form a thick paste. The mill is then rotated until the fluid is well incorporated—and then more saline is added—and the mill again rotated until the mixture has the consistency of a thin paste. This paste may then be considerably diluted, and the mill rotated until the clumps of bacilli are finally broken up, and a suspension formed which will not sediment for several hours. It usually requires about ten hours to make a satisfactory suspension of 50 grams of dried bacilli. If too much saline is added at the beginning some of the dry powder is entrapped in the froth, remains floating on the surface, and increases the difficulty of making a homogeneous suspension. The suspension is finally diluted to contain 5 mg. per c.c., and is dispensed into 100 c.c. bottles which are closed with rubber caps.

Dosage of Vaccine.

The first dose of vaccine is given as soon as practicable after the calf is born, and the next three doses at intervals of one to four weeks. It is, of course, not practicable for the veterinary surgeon to make a special visit to inject each calf the day it is born; but he calls at frequent intervals, and injects several animals at the same time. The age of the calves at the time the first dose is given has therefore varied from one day to several weeks. The intervals between the first, second, third and fourth dose also vary considerably—depending largely on the convenience of the veterinary surgeon.

After this preliminary series of doses, the calves receive their subsequent vaccine together with the rest of the herd. When the animals are brought into the stable from the fields or meadows in October or November, and again before being turned out to graze in April or May, each animal is tuberculin tested, and when the

last reading of the tuberculin test is made a dose of vaccine is given, followed by another one in two to five weeks. It occasionally happens that an animal is turned out to graze before the date of the tuberculin test, or is left out in the meadows when the rest of the herd is brought into the stables. Such animals have missed the routine tuberculin test and dose of vaccine.

The majority of the injections have been given intravenously. It has been found, however, that pregnant cows do not always tolerate large doses well, and during the later part of the experiment all cows in which pregnancy could be determined by rectal examination, *i.e.*, all in which pregnancy was advanced three months or more, were injected intraperitoneally, in the right flank, with a dose slightly larger than would otherwise have been given intravenously.

As a general rule, the first four doses have been, 1·5 c.c., 3 c.c., 4·5 c.c., and 6 c.c. respectively of the vaccine (5 milligrams per c.c.). If the animals were several months old when first vaccinated—as in the case of the heifers and bulls brought from other places—the first four doses have been slightly larger, *viz.*, 2 c.c., 4 c.c., 6 c.c., and 8 c.c. respectively. At subsequent injections the doses were increased gradually up to 30 or 35 c.c. We considered that these doses were probably unnecessarily large, and decided in 1928 to reduce them. The maximum dose for intravenous injection is now 10 c.c., and the maximum for intraperitoneal injections 15 c.c. There seems to be no evidence that these smaller doses are less effective than the larger doses previously given.

In future we propose to give the first course of four doses intravenously and all subsequent doses intraperitoneally.

Accidents of Vaccination.

It may frequently happen that the calves, when they receive intravenously the third or fourth injection of 4·5 c.c. or 6 c.c., show some sign of anaphylactic shock. The calf stands for a moment, swaying from side to side, and then suddenly falls on its side, and may give the impression that it is on the point of death. If the calf is immediately lifted up and slapped on the side of the thorax it quickly recovers, but may have a slight cough with shortness of breath and rapid superficial respiration, which may last for a few hours. Some animals appear to be much more sensitive than others, and may show similar symptoms on several different occasions after injections. The symptoms are readily relieved by the injection of adrenalin. All these symptoms may be prevented entirely by making the animal run about the room a few times immediately after the injection.

Four animals have died as a result of intravenous injections, Nos. 4, 45, 252 and 307.

No. 4 had been tuberculous at the beginning of the experiment

and had been treated with sanocrysin until the tuberculin reaction had become negative. The initial dose of vaccine was given at the age of 16 months. In all, 60 c.c. of vaccine in six doses had been given. The animal was by this time in an advanced stage of pregnancy. The seventh dose, 20 c.c. intravenously, produced a severe reaction, and the animal died from œdema of the lungs. This occurred in an early period of the experiment, before the staff were aware of the possibility of reactions, and the attention of the veterinary surgeon was only called to the case when the animal was dying.

No. 45 had been inoculated at regular intervals over a period of eight years. A dose of 10 c.c. was given intravenously April 28th, 1933, and it showed no symptoms of anaphylactic shock immediately after the injection. The next day it was rather short of breath, but the heart seemed to be normal. Forty c.c. of a 20 per cent. solution of camphor in olive-oil was injected subcutaneously. The animal died during the course of the following night. *Post-mortem* there was extensive œdema of the lungs and the heart was dilated. There was no sign of tuberculosis.

No. 252 received an initial dose of 1·5 c.c. intravenously at the age of 54 days. The second dose of 3 c.c. 56 days later caused an anaphylactic reaction resulting in œdema of the lungs, and the animal died the same day.

No. 307 was a small and weak calf at birth. It was given a first dose of 1·5 c.c. when nine days old, and a second dose of 3 c.c. 42 days later. Œdema of the lungs developed, and in spite of treatment with Ephedrin (Merck), it died two days later. *Post-mortem* there was œdema of the lungs. There was no sign of tuberculosis. The rather long interval between the first and second dose may possibly account for the hypersensitivity which has developed in Nos. 252 and 307.

Pregnant animals are particularly sensitive to the intravenous injection of vaccine, and tend to develop a cough, rapid, superficial respiration, and tachycardia. They were treated when necessary with subcutaneous injections of adrenalin and camphor. In order to avoid these rather severe reactions, during the later part of the experiment pregnant animals have usually been injected intraperitoneally.

No serious anaphylactic reactions have developed as a result of the intraperitoneal injections. One cow, No. 11, which had been tuberculous at the beginning of the experiment and had been treated with sanocrysin until the tuberculin reaction had become negative, was first vaccinated at the age of 14 months. It received in all twelve injections from February 25th, 1925 to May 12th, 1928. Peritonitis, due to some undetermined secondary infection developed after the injection of 30 c.c. of vaccine on May 12th, and the cow died on May 16th. *Post-mortem* there was no sign of tuberculosis.

Tuberculin Tests.

The tuberculin used for the majority of the tests was obtained from the Royal Veterinary High School Serum Institute (Professor C. O. Jensen), Copenhagen, but for some of the tests the tuberculin was kindly supplied by Professor J. B. Buxton, F.R.C.V.S., of Cambridge. The technique employed was that usually followed in Denmark. An area of skin on the neck is clipped, and swabbed with spirit. A fold of the skin is then pinched up between the thumb and forefinger, and 0·1 c.c. of tuberculin is injected intradermally. The thickness of the fold of skin is measured with a pair of callipers immediately before the injection. Subsequent measurements are made in an exactly similar manner 48 and 72 hours after the injection. At the same time the other signs of the reaction are observed.

Since November, 1925, all tuberculin tests have been made by one of us (H. H. A.), who had had great experience with the technique. The personal factor was therefore constant throughout. The measurements were dictated as they were made, and the observer did not know what his previous measurements had been for any animal.

A positive reaction is indicated by an increase in the thickness of the skin fold, with œdema, hyperæmia, pain or tenderness, and increase in temperature at the site of injection, although any of these signs may be absent.

The increase in thickness of the skin fold lends itself most readily as a basis for the comparison of the degree of reaction in different animals, or in the same animal at different times. As a general rule, therefore—particularly in the early part of the experiment, we have arbitrarily considered an increase of 2·5 millimetres or more in the skin fold thickness as a positive reaction, but this rule could not be rigidly followed at all times. In certain cases where the increase in thickness was less than 2 millimetres, it was obvious from the hyperæmia, tenderness, and increased temperature of the site of injection that the reaction was positive. In other cases, where the increase in thickness was somewhat more than 2·5 millimetres, the absence of all the usual accompanying signs excluded a positive reaction.

The tuberculin reaction is generally accepted as a reliable method of diagnosis of tuberculous infection in cattle. When applied to immunised animals, however, its value as a diagnostic agent is considerably decreased, since sensitivity to tuberculin may develop as a result of the vaccination. It has been shown that guinea-pigs treated with a vaccine similar to the one used in this experiment become sensitive to tuberculin (Dreyer and Vollum, 1931). There is now strong evidence that cattle show the same phenomenon. In many cases immunised animals which have reacted very strongly to tuberculin on several occasions have subsequently become negative to tuberculin on one or more

occasions. These animals have shown no clinical evidence of tuberculous infection, and several which have been examined *post-mortem* have been found quite free of tuberculosis.

After considerable experience in examining the tuberculin reaction of vaccinated and infected animals respectively, we have come to the conclusion that, while it is impossible to differentiate absolutely between a reaction due to infection and a reaction due to vaccination, the reactions tend to differ in some respects. In a reaction due to infection, the site of injection is hyperæmic, warm, and tender, and the thickness of the skin fold is increased. This increase in thickness persists for three to five days, or more, and the 72 hour measurement is usually the same or somewhat higher than the 48 hour measurement. In the reaction due to vaccination, on the other hand, the site of injection tends to be less warm, and even if swelling is considerable, the tenderness is less than in the infected animals. Furthermore, the swelling tends to decrease more rapidly, so that the measurement of the skin fold at 72 hours is frequently less than at 48 hours.

The Non-Experimental Group.

The 142 animals which reacted positively to the tuberculin test September 30th, 1924, constitute the greater part of the non-experimental Group. For purposes of reference they have been numbered serially from 01 to 0142. As they were all tuberculous when tested September 30th, 1924, and as no treatment of any kind was applied, it may be assumed that each one was a potential source of infection as long as it remained in the herd. The majority of these animals have now disappeared from the herd, having died, or having been sold. The information about these is scanty, but it is known that at least two have died of foot-and-mouth disease. Seven have died from other diseases, and at least seven have died from, or been killed on account of, tuberculosis. The number remaining in the herd at September 30th each year since the experiment started is as follows :—

Sept. 30th, 1924 ...	142	Sept. 30th, 1929 ...	20
,, 30th, 1925 ...	107	,, 30th, 1930 ...	11
,, 30th, 1926 ...	83	,, 30th, 1931 ...	10
,, 30th, 1927 ...	47	,, 30th, 1932 ...	8
,, 30th, 1928 ...	29		

The survivors were again tested with tuberculin October 27th, 1931, and at the three later general tuberculin tests, and all were strongly positive on each occasion. These non-experimental animals may be considered as the chief source of infection in the herd. The offspring of these animals constitute a large part of the experimental group.

In addition to these animals which were in the herd at the beginning of the experiment, 13 heifers which were bought, and not vaccinated, may be considered together with the non-experi-

mental group, of which they form Nos. 0143 to 0155 inclusive. Nos. 0143 to 0153 inclusive were purchased in August, 1926, as two-year-old heifers. These were not tuberculin tested before being bought. Nos. 0154 and 0155, reacted positively when bought in January, 1927. The survivors of these animals were tuberculin tested October 27th, 1931, and at the three later general tuberculin tests, and all were strongly positive on each occasion.

At the time of writing (July, 1933), there are still 13 survivors of this Non-Experimental Group, which remain as a potential source of infection in the herd.

Treated Animals. (*See* Table 3.)

1.—*Vaccine Treated.*

Three experimental animals were not vaccinated until after they had become tuberculin positive as a result of naturally acquired infection. They have therefore been classified as "Vaccine Treated"—but no attempt was made to carry out a special course of vaccine therapy. The animals were merely injected with the vaccine in the same doses and at the same times as the normals in the hope of producing enough immunity to stop the spread of the infection.

Nos. 1 and 13 were positive when first tested September 30th, 1924, and remained positive as long as they were in the herd. No. 1 never developed any sign of clinical tuberculosis. It was sold on account of œdema of the uterus. No. 13, a bull, became sterile, and was sold. The clinical diagnosis was tuberculosis of the testes. No *post-mortem* details are available.

No. 38 was originally left as a control, and was positive when first tested at eleven months. When it was 34 months old, vaccination was started, and continued until the animal was killed at 69 months on account of sterility. When last tested, six months before it was killed, the tuberculin reaction was negative. It had been a good milk producer for three years, but had last calved December 25th, 1927, and since then had been sterile. The animal was in excellent condition. It was killed for beef. *Post-mortem* there was one calcified inguinal gland, and the bronchial glands were calcified. One half the pleura costalis on one side was covered with large nodules, extremely calcified, like marbles. There was absolutely no sign of fresh tuberculosis. The negative tuberculin reaction and the *post-mortem* findings provide evidence that in this case the vaccine had some definite therapeutic effect.

Sanocrysin Treated Animals.

Nine experimental animals were treated with sanocrysin. These were all young animals which were strongly positive to the tuberculin test. As a result of the treatment, the intensity of the tuberculin reaction was considerably reduced in all except one of these animals, but only two became completely negative.

2.—*Sanocrysin and Vaccine.*

Four of the sanocrysin treated animals, including the two which were negative to the tuberculin test after treatment, were subsequently vaccinated, *viz.*, Nos. 4, 11, 12 and 44.

No. 4.—The tuberculin reaction had become negative as a result of treatment, but became positive again after vaccination. A large dose of vaccine injected intravenously while this animal was in an advanced stage of pregnancy, produced a fatal anaphylactic reaction, death being due to œdema of the lungs. *Post-mortem* there was no sign of tuberculosis.

No. 11.—Became negative to the tuberculin test after sanocrysin treatment. At this time it was 14 months old. Vaccination was then started, and the tuberculin test again became positive. The test was positive on four occasions, at the age of 18, 23, 29 and 33 months respectively. At the age of 40 and 46 months respectively the reaction was negative. When tested again at 52 months, the reaction was positive. About one month later the animal died from peritonitis subsequent to an intraperitoneal injection of vaccine. *Post-mortem* the peritonitis was found to be due to a secondary infection. There were no macroscopic tuberculous lesions.

No. 12.—The tuberculin reaction in this animal had become almost negative as a result of the sanocrysin treatment, and vaccination was started. The tuberculin reaction became more strongly positive again, and remained positive throughout. This cow was killed for beef, because its milk production was unsatisfactory. No macroscopic tuberculosis was found *post-mortem.*

No. 44, was originally left as a control, and as a result of naturally acquired infection reacted positively to the tuberculin test at four months. Sanocrysin treatment was carried out during the next month, but the tuberculin reaction did not become negative. Vaccination was started after the course of sanocrysin was finished. The tuberculin reaction remained positive throughout. The animal was eventually slaughtered because of sterility. It was in excellent condition, and very fat. There was a soft, fresh tuberculous process in the left bronchial gland, and an old calcified, healed lesion in the mediastinal gland. There was no other sign of tuberculosis.

3.—*Sanocrysin—Not Vaccinated.*

In Nos. 6, 7, 10, 16 and 18 the tuberculin reaction was still definitely positive after the sanocrysin treatment, although in each case the intensity of the reaction was very much less than before. None of these animals were vaccinated. They were all eventually sold on account of sterility. *Post-mortem* examinations were made only of No. 16 and No. 18, and in both of these fresh tuberculous lesions were found.

The two groups of sanocrysin treated animals, one of which subsequently received vaccine, are not strictly comparable, as two of the four vaccinated animals had become negative to the tuberculin test, and a third one was only weakly positive, whereas none of the non-vaccinated animals had become negative as a result of treatment. It may be mentioned, however, that three of the four

vaccinated animals were free of tuberculous lesions, *post-mortem*, at ages of 26, 53 and 65 months respectively. The other had one fresh lesion in a bronchial gland. Two non-vaccinated animals, examined *post-mortem* at 42 and 31 months of age respectively, both had fresh tuberculous lesions.

Controls.

As mentioned above, when the experiment was planned it was intended that every third calf should be left as a control, and the other two vaccinated. However, about seven months after the experiment started, the vaccinated animals appeared to be in better condition than the controls, and the owner of the herd then requested that all the calves subsequently born should be vaccinated. During the first seven months 15 animals were left as controls, but two of these (Nos. 38 and 44) were treated after the tuberculin reaction had become positive, and they are therefore included among the treated animals. In addition to the 13 remaining animals, one calf born in 1932, which was tuberculin tested but never vaccinated, may also be considered among the controls. Details of these controls are given in Table 4.

It will be seen from Table 4 that 12 of the 14 controls were negative to tuberculin when first tested. One of these, No. 9, was still negative when last tested at 23 months. It was sold six months later, after an attack of foot-and-mouth disease, and no further information is available. The ages of the other eleven animals when last negative to the tuberculin test were 18, 15, 14, 13, 13, 12, 9, 9, 8, 6 and 5 months respectively. Nos. 53 and 321 were positive when first tested at the age of six months and 18 days respectively.

It will be observed that, as is to be expected, none of the controls which became positive to the tuberculin test has ever been negative at any subsequent test.

Two of the controls are still alive. Twelve have died, or have been killed, or sold, at ages varying from one to 115 months. *Post-mortem* examinations have been made on seven of these, and in each case fresh tuberculous lesions were found.

No. 30 died of tympanites. Fresh tuberculous lesions were found in the lungs.

No. 34 had to be killed on account of spinal tuberculosis. Generalised tuberculosis with extensive lesions in the spinal column was found *post-mortem*.

No. 47 was killed for experimental purposes. The animal was somewhat thin. There were fresh tuberculous lesions in the bronchial glands, and two superficial tubercles in the lung. Other organs and glands were normal.

No. 321 gave a very strong positive tuberculin reaction when tested 18 days after it was born. It was killed five days later, and acute tuberculous processes were found in the portal glands. The mother of this calf was one of the original non-experimental cows, No. 0127,

which reacted positively September 30th, 1924, and was never vaccinated. This animal reacted very strongly to tuberculin when tested at the times of the last four general tuberculin tests. Material from the uterus was examined, but no *B. tuberculosis* was found. Through an oversight, the calf had been allowed to suck the mother for five or six days, and was probably infected through the milk.

Animals Bought—Immunised.

During the course of the experiment a number of animals have been purchased to replace losses due to sterility, infectious abortion, etc. Thirty of these have been considered in a special group as the details of their history, exposure to infection, etc., before coming in to the herd are not known. All 30 animals were vaccinated, 15 of them within six weeks of coming in to the herd; eleven after six months; and four only after nine or ten months. All were tested at the times of the regular tuberculin tests. The results of these tests, and other details may be seen in Table 5.

Thirteen of the 30 reacted positively when first tested. The interval between coming into the herd and the tuberculin test depended on the date they were bought, but varied up to nine months. One of the 13 was first tested and found positive after nine months, one after seven months, one after five months, and nine after six months. No. 134 was tested the day it came into the herd and was positive. It was disposed of 17 months later on account of clinical tuberculosis. *Post-mortem,* there were extensive fresh lesions. Nos. 130 and 212 had not been vaccinated until after their first positive test, and the reaction must therefore be considered to be due to infection acquired either before or after coming into the herd. The other nine which reacted positively when first tested had received doses totalling 14 to 48 c.c. of vaccine before the test, so in these cases the positive reaction may be ascribed either to infection or to immunisation. In Nos. 142G and 142L the positive reaction was most probably due to immunisation, as the reaction was negative at a later test. No. 142F was positive on each of the three occasions it was tested, but when killed for beef because of sterility there was no sign of tuberculosis *post-mortem*. No. 109, a bull, which was killed on account of sterility, was found *post-mortem* to have extensive pulmonary tuberculosis.

Of the 17 animals which were negative to the first tuberculin test after coming into the herd, Nos. 210 and 219 remained negative for the comparatively short time they were in the herd—(14 and 18 months respectively when last tested)—and No. 210 was free of tuberculosis *post-mortem*.

The other 15 all developed a positive reaction later, but seven of these were negative at one or more subsequent tests, so in these cases the positive reactions were probably a result of the vaccination. Nos. 211 and 217, which did not again become negative to tuberculin, were found *post-mortem* to be free of tuberculous

lesions. No. 211 was killed because of sterility, and No. 217 because of mastitis.

Vaccine Group.

The Vaccine Group consists of 281 animals, of which eight were negative to the tuberculin test September 30th, 1924, and were selected for immunisation; 266 were born in the herd since then, and have been regularly vaccinated from an early age; seven (Nos. 180, 181, 182, 183, 185, 186, 259) were purchased as very young calves from a neighbouring estate and were vaccinated as soon as possible. Table 6 shows the date of birth, time in herd, age when first vaccinated, results of tuberculin tests and the *post-mortem* results of these animals.

The preparation and dosage of the vaccine has been described above. The age, in days, when the first dose was given is shown in Table 6.

43·5 per cent. have received the initial dose in the first week; 21·7 per cent. in the second week; 12·8 per cent. in the third week; 9·6 per cent. in the fourth week; 5 per cent. in the fifth week; and 7·5 per cent. later than the fifth week.

An important factor which must be considered in estimating the results of the immunisation is, of course, the exposure to infection. It has already been mentioned that no attempt is made to isolate the infected animals, and that there is opportunity for the spread of infection both through feeding and by direct contact throughout the whole time any animal is in the herd. The chance of infection, however, must have been much greater at the beginning of the experiment, when 90 per cent. of the animals in the herd were definitely known to be tuberculous, than it is at the present time, if the immunisation has been effective. It may be seen from Table 2 that the number of known infected non-experimental animals, which are the chief source of infection in the herd, has been progressively decreasing throughout the course of the experiment. The infected controls and treated animals, as well as any immunised animals which have become infected, also serve as sources of infection. Apart from this variation in the number of infective animals, there is doubtless an enormous variation in the actual amount of infective material acquired by the individual immunised animals.

In a practical field experiment, where conditions are constantly changing, it is difficult to form an accurate estimate of the result. The immunising effect of the vaccine must be assessed chiefly on the results obtained in this group of 281 animals, which have been under observation from the time they were born.

As a basis for judging the effect of the vaccine we may consider the evidence of (1) the *post-mortem* examinations, (2) the tuberculin test, and (3) the clinical condition of the animals.

1.—*Post-mortems.*

One hundred and seven of the 281 animals in this group have been killed, or sold, or have died, and *post-mortem* examinations have been made on 55 of the 107, *i.e.*, 51·5 per cent. The ages of these animals vary from one to 98 months. No tuberculous lesions of any kind were found in 49 of the 55 animals (89 per cent.). Calcified healed lesions were found in three (5·5 per cent.); fresh processes in a bronchial gland in one (1·8 per cent.). Acute advancing tuberculous lesions were found in the remaining two (3·6 per cent.). Three of the six animals in which tuberculous lesions were found *post-mortem* were born in January, 1925, when 82 per cent. of the herd were definitely known to be tuberculous, and the chance of infection, therefore, was much greater than it is at present. One was born in 1927, one in 1928, and one in 1930. Four of the six were born of infected mothers which reacted positively to tuberculin September 30th, 1924. One was from a purchased cow which has reacted positively to tuberculin throughout; and one from an immunised cow. These last two had lesions in the bronchial glands only. It is possible that the four, three of which had extensive lesions, may have been infected from their mothers, if through an oversight they happened to have been allowed to suck. However, it must not be assumed that the offspring of tuberculous mothers are likely to be infected, since 33 of the 49 animals (67·5 per cent.) which were found to be free of tuberculosis *post-mortem* were from mothers definitely known to be tuberculous.

Post-mortem Notes on Vaccine Group.

No. 39.—Killed for experimental purposes. Tuberculous lesions in portal and mediastinal glands, spleen, and kidneys, all greatly calcified. No tuberculosis in lungs.

No. 40.—Killed for experimental purposes. Animal fat, and in good condition. One highly calcified, apparently healed tubercle in left bronchial gland. Otherwise all organs and glands macroscopically normal.

No. 42.—Tubercle in portal gland; isolated miliary tubercles in lungs; extensive fresh tuberculosis in peritoneum and pleura; marked tuberculous meningitis.

No. 70.—Killed for experimental purposes; no macroscopic tuberculosis.

No. 77.—Animal in good condition; no macroscopic tuberculosis.

No. 126.—No macroscopic tuberculosis.

No. 139.—One calcified tubercle size of small split pea in bronchial gland. No other macroscopic tuberculosis.

No. 162.—Clinically diagnosed as acute tuberculosis of uterus. *Post-mortem,* tuberculous broncho-pneumonia; tuberculous lesions in pleura and peritoneum; all processes soft and fresh without any sign of healing.

No. 170.—Died as result of accident. Stick penetrated udder.

Extensive subcutaneous inflammation over abdomen. No macroscopic tuberculosis.

No. 180.—Slaughtered for beef. No macroscopic tuberculosis.

No. 192.—Slaughtered for beef. Poor milk producer. No macroscopic tuberculosis.

No. 197.—Slaughtered for beef. Poor milk producer. No macroscopic tuberculosis.

No. 223.—Slaughtered for beef. No macroscopic tuberculosis.

No. 225.—Died from typical acute broncho-pneumonia. No tuberculosis, confirmed by microscopical examination.

No. 244.—Vaccinated by mistake intravenously when pregnant March 27th, 1933. Aborted March 29th, 1933. After this not well, and died May 28th, 1933.

Post-mortem, anæmic; marked catarrh of intestinal mucosa. In left bronchial gland five or six (?) tuberculous processes less than a small pea in size. No calcification. (Not confirmed microscopically.)

No. 252.—Œdema of the lungs subsequent to vaccination.

No. 307.—Small and weak calf when born. Œdema of lungs after vaccination November 19th, 1932. Treated with ephedrin, Merck. Died November 21st, 1932. *Post-mortem,* œdema of lungs. No macroscopic tuberculosis.

No. 315.—Had been ill and had lost weight. *Post-mortem,* gall bladder and bile ducts acutely inflamed; gall bladder six to eight times normal size, thick walled and œdematous; contained foul smelling pus. No macroscopic tuberculosis.

2.—*Tuberculin Tests.*

It has been pointed out above that the validity of the tuberculin test as a criterion of tuberculous infection is considerably impaired by the process of immunisation. The tuberculin test may be positive as a result of a tuberculous lesion, or as a result of the vaccination, or as a result of a combination of the two factors. So long as an active lesion persists, the tuberculin reaction will probably remain positive. If the lesions heal, the tuberculin reaction may, in time, become negative. (The reaction may become negative also in cases of very advanced clinical tuberculosis.)

In view of the conditions of this experiment, it is apparent that all animals have been exposed to infection, and it is probable that some of the immunised animals may have had a small lesion in a bronchial or mesenteric gland which has healed without having caused any symptoms. Such lesions may account for some of the positive tuberculin reactions in the immunised animals, but we consider that the majority of the positive reactions are due to vaccination.

The tuberculin reactions of each animal are shown in Table 6. On examination of this table it will be found that all vaccinated animals have reacted positively to tuberculin by the time they are three years old. Many of these have been negative at one or more subsequent tests; but all animals which have been negative to tuberculin at ages of 36 months or more have been positive at one

or more previous tests. Table 7 shows the number of animals which had been in the herd for periods of three to eight years during the course of the experiment and the percentage of these which had become negative to tuberculin on one or more occasions after having been positive. Thus it may be seen that 134 animals have been in the herd for at least 36 months, and 72 (53·7 per cent.) of these have been negative after having been positive. This percentage gradually increased in the older groups, and all of the eight immunised animals which have been in the herd for at least 96 months have been negative after having been positive.

In Table 8 is shown the distribution in various age groups of the vaccinated animals, with the number of positive and the number and percentage of negative reactions in each group at each test separately, and also for all tests together during the experiment. It will readily be seen from the figures for all the tests together, that the percentage of negative reactors falls steadily to a minimum in the age group 37 to 48 months, and then rises regularly in the older groups. In the oldest group—97 to 103 months— 81·8 per cent. of the tuberculin reactions were negative.

Tables 7 and 8 show clearly that the proportion of positive reactions in immunised animals increases with increasing age up to three or four years, and after that time steadily decreases. If these positive reactions were due to infection, the proportion of positive reactions would remain the same, or would increase, rather than diminish, in animals over the age of four years. On the other hand, if the positive reactions were due to vaccination, the decrease in intensity and the eventual disappearance of the reaction, and also the reappearance after further immunisation, would be in accord with general experience of immunity reactions in vaccinated animals.

We are convinced, therefore, that in the great majority of cases, the positive reaction to tuberculin is the result of vaccination, and is not due to infection.

The thesis that positive reactions in immunised animals are usually due to the vaccine and not to infection is also supported by a consideration of the tuberculin tests of the animals which have been examined *post-mortem*. *Post-mortem* examinations have been made on 55 vaccinated animals, and in 49 of these there was no sign of tuberculosis. The last tuberculin test in 24 of these 49 was positive.

As a typical example of the sensitivity to tuberculin in immunised animals, the measurements of the tuberculin reactions of No. 127 are given in Table 9. The total amount of vaccine given in the intervals between the tuberculin tests is also shown. No. 127 was born March 28th, 1927; the first dose of vaccine was given when the calf was two days old. The first test was negative, the second positive, and at the third—when 20 months old—the animal had become very sensitive and the reaction was intense.

After this the intensity of the reaction decreased. No vaccine had been given between April 19th, 1930, and April 24th, 1931, as an epidemic of foot-and-mouth disease occurred after the tuberculin test in November, 1930, when the vaccine would normally have been given. Probably as a result of the long interval between doses, the reaction was negative April 22nd, 1931, at the age of 49 months, and did not become positive again until November 16th, 1932. The animal died three months later as a result of severe hæmorrhage during calving. There was no sign of tuberculosis *post-mortem,* although the last tuberculin reaction had been positive.

3.—*Clinical Notes.*

At the time the experiment began, it was apparent, even from a very superficial examination, that tuberculosis was very widespread in the herd, and that there were many serious cases of open tuberculosis. During each previous year there had been two or three cases of cerebral tuberculosis or tuberculous meningitis among the calves and heifers. These cases had become so common that even the farm hands had become familiar with the clinical signs, and could diagnose them before the veterinary surgeon arrived to examine the case, or to make the *post-mortem* examination. Tuberculosis of the uterus was also very common, and was frequently discovered in the course of the routine examinations *per rectum* made four or five times each year either to detect pregnancy, or in the treatment for sterility. Tuberculosis of the udder was also not uncommon.

Since the beginning of the experiment, among the vaccinated animals there has been only one case of tuberculous meningitis (No. 42), and only one case of tuberculosis of the uterus (No. 162). With the exception of these two cases there has been no clinical tuberculosis among the vaccinated animals.

During the period of the experiment there have been four severe epidemics of foot-and-mouth disease. These epidemics started on December 17th, 1924; March 31st, 1926; May 27th, 1930 and December 4th, 1930, respectively, and lasted for four to six weeks. Practically all animals in the herd were attacked in these epidemics, and the last was particularly severe. As a general rule, the feet were more seriously affected than the mouths of the cattle.

It is a common experience that a latent tuberculous infection may become active and develop rapidly as a result of the lowered resistance during or subsequent to an acute infection such as foot-and-mouth disease. In the periods immediately following the above-mentioned epidemics, the number of non-vaccinated animals which had to be disposed of on account of tuberculosis was greater than usual, whereas none of the vaccinated animals have shown any symptoms of tuberculosis following an attack of foot-and-mouth disease,

In general, the vaccinated animals have been in excellent condition throughout the course of the experiment.

Discussion.

Bovine tuberculosis has long been an all too familiar disease in most European countries. As a result of long experience, it is generally accepted as a fact that in an infected herd tuberculosis does not tend to die out, but, on the contrary, continues to spread and increase unless some method such as the segregation of the infected animals is adopted to prevent it. In recent years the tuberculin test has been extensively used as a method of diagnosis of tuberculosis in cattle. The extensive experience of numerous veterinary surgeons has established the fact that an infected animal will continue to react positively to tuberculin throughout its life—except, at times, in the terminal stages of the infection—if no measures are taken to treat the disease.

In considering the results of our experiment, we have assumed that these two facts are correct.

The records of two herds of cattle from other estates (V and H) on the island of Lolland provide additional evidence as to the correctness of these facts. Each herd numbers about 100 head of cattle, which are kept under conditions similar to those at Söllestedgaard where our experiment was carried out. No method of immunisation has been attempted with these animals, but the younger ones are isolated to some extent from the older stock. We have been permitted to carry out tuberculin tests on these herds in 1929, 1931 and 1933. In herd V, all cows were positive at each test. In herd H, 79 per cent. of the cows were positive in 1929, and 100 per cent. were positive in 1931 and in 1933. In no case did any animal give a negative tuberculin reaction subsequent to a positive reaction. From the records of these two herds during the last four years there is no evidence of any tendency for bovine tuberculosis to decrease spontaneously in an infected herd.

As previously stated, the number of controls is unfortunately limited—and furthermore—all but one of them were born at a time when the proportion of tuberculous animals in the herd was still very high. They cannot, therefore, be considered as fair controls to the vaccine group, but they may be fairly compared with the treated animals which were born about the same time. *Post-mortem* examinations have been made on seven controls, and fresh tuberculous lesions were found in all. None of the controls has ever been negative to tuberculin after having been positive. One of the three infected animals treated with vaccine only eventually became negative to tuberculin, and was found *post-mortem* to have extensive healed, calcified lesions, and no fresh lesions. Three of the four treated with sanocrysin and subsequently vaccinated were free of tuberculosis *post-mortem*, and one

of these had become negative to tuberculin after treatment. The other, which had only a short course of sanocrysin treatment, had fresh and calcified lesions *post-mortem*.

A considerable degree of immunity has apparently been developed in four of these seven treated animals. The five animals treated with sanocrysin only, and not subsequently vaccinated, never became completely negative to tuberculin—and were naturally exposed to infection after the treatment. Fresh tuberculous lesions were present in the two examined *post-mortem*. The benefit which was probably obtained from the sanocrysin treatment could not be maintained without subsequent vaccination.

The success of the method of immunisation we have used must be judged chiefly on the result obtained in the Vaccine Group, which now constitutes the greater part of the herd. At the last tuberculin test, March 25th, 1933, there were 186 animals in this group—17 more than in the whole herd at the beginning of the experiment, September 30th, 1924.

A comparison of the results of the first and last tuberculin tests (September 30th, 1924, and March 25th, 1933) shows a marked difference. At the first test, only 17 of 169 animals were negative to tuberculin. Of these 17, ten were from one to six months, six were from seven to twelve months, and one was 13 months old. At the last test 104 of 186 vaccinated animals were negative to tuberculin. Of these, 30 were from one to six months, seven were from seven to twelve months, and 67 were older than twelve months.

These figures show that a very considerable improvement has taken place during the course of the experiment. But the evidence available from the *post-mortem* examinations, and from the alternation of positive and negative reactions in immunised animals, shows that a very high percentage of the positive reactions in these animals is due to the vaccination. The positive tuberculin reaction in the immunised animals does not, therefore, indicate infection except in a small percentage of cases. This is also shown by the clinical records. During the course of the experiment there have been only two cases of clinical tuberculosis among the 281 immunised animals. Tuberculous lesions which had never produced any symptoms were found in four immunised animals *post-mortem*. In 55 *post-mortem* examinations (including the two clinical cases) tuberculous lesions were found in six cases (10·9 per cent.).

Conclusions.

In a practical field experiment extending over a period of nine years, on a herd of cattle which was originally highly infected with tuberculosis, the calves born in the herd, and a few which have been bought in, have been regularly vaccinated with a dead vaccine consisting of " defatted " tubercle bacilli (diaplyte vaccine). By this means it has been possible, without isolation or segregation of

the immunised animals, to produce and maintain a high degree of immunity to tuberculosis. Ninety per cent. of the animals in the herd were tuberculous at the beginning of the experiment, and although the immunised animals were allowed to mix freely with the infected stock, there have been only two cases of clinical tuberculosis among the 281 animals which have been immunised from an early age. Out of 55 animals which were examined *post-mortem,* six were found to have tuberculous lesions, and in three of these the lesions were all calcified. If the animals which have been examined *post-mortem* be considered as a sample of the immunised group, they would indicate that only 10·9 per cent. of the Vaccine Group have become infected, whereas at the beginning of the experiment 90 per cent. of the herd were infected.

The procedure adopted is such that it could be applied to any herd of cattle without undue inconvenience or interference with the commercial management of the herd.

We wish to express our grateful thanks to Mr. Theophil Jörgensen for permitting us to carry out this experiment on his herd of cattle, and for generously affording us all the facilities for our work; and to Mrs. Ebba Jörgensen for her invaluable assistance in keeping the records for us. We also wish to thank Professor Major Greenwood, F.R.S., for his advice on certain points after he had kindly examined the records of our experiment at the end of the first seven years. One of us (R. L. V.) is indebted to the Medical Research Council for a part-time grant.

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TABLE 1.

The Composition of the Herd at the Beginning of the Experiment 30.9.1924.

- Milch Cows 105 — All Tuberculin Positive (Non-Experimental Group)
- Calves, Heifers, Bulls 64
  - 37 Tuberculin Positive (Non-Experimental Group)
  - 10 Tuberculin Positive
    - 8 Sanocrysin Treated
      - 3 Later Vaccinated
      - 5 Not Vaccinated.
    - 2 Vaccine Treated.
  - 17 Tuberculin Negative
    - 9 Controls.
    - 8 Vaccinated.
- Total 169
  - 142 Non-Experimental.
  - 27 Experimental.

TABLE 2

NUMBERS OF ANIMALS OF VARIOUS GROUPS IN HERD AT TIMES OF TUBERCULIN TESTS.

DATE OF TEST	NON-EXPERIMENTAL. No. 01 to No. 0142	No.0143 to No.0155 (Bought)	TOTAL NON-EXPERIMENTAL.	EXPERIMENTAL. TREATED WITH SANOCRYSIN	SANOCRYSIN and VACCINE.	VACCINE.	BOUGHT-VACCINATED	CONTROLS	VACCINE GROUP.	TOTAL EXPERIMENTAL.	TOTAL ANIMALS IN HERD.
30/9/24	142		142	5	3	2		9	8	27	169
25/11/24	135		135	5	3	2		11	12	33	168
31/1/25	126		126	5	3	3		11	19	41	167
20/4/25	119		119	5	4	3		13	27	52	171
4/8/25	117		117	5	4	3		13	28	53	170
3/11/25	106		106	5	4	3		13	35	60	166
11/5/26	92		92	5	3	2		11	59	80	172
6/9/26	85	11	96	5	3	2		11	64	82	178
16/4/27	60	12	72	3	3	2	5	7	88	108	180
16/10/27	47	12	59	2	3	2	17	5	88	117	176
18/4/28	42	11	53	1	2	1	18	5	99	126	179
27/11/28	29	9	38	1	1	1	18	5	106	132	170
22/3/29	20	8	28		1	1	24	5	116	147	175
7/11/29	20	7	27			1	25	3	120	149	176
18/3/30	17	7	24			1	25	3	125	154	178
12/11/30	11	7	18				24	3	135	162	180
22/4/31	10	7	17				18	3	144	165	182
27/10/31	10	6	16				17	3	150	170	186
12/4/32	10	6	16				15	3	172	190	206
16/11/32	8	6	14				12	4	184	200	214
25/3/33	8	5	13				10	3	186	199	212

TABLE 3
TREATED ANIMALS.

TREATED WITH	EXP. No.	BORN	OUT DATE	OUT AGE MONTHS	SANO-CRYSIN GRAMS GIVEN	DATE FIRST DOSE VACCINE	TUBERCULIN TESTS. 1924	1925	1926	1927	1928	1929	1930	REMARKS. S.= Sold. K.= Killed D.= Died. * = Details in Post Mortem Notes.	Post Mortem TB Lesions O or +
VACCINE	1	13/5 23	2/12 25	32		2/12 24	+ +	+ + +						S. Oedema of Uterus.	
"	8 13	1/1 24	3 28	50		11/3 25	+		+ + +					S.* Tb of testes.	
"	38	13/12 24	23/9 30	69		23/10 27			+ +	+ +	+ +	+ +	O	K.* Calcified Tb. lesions	+
SANOCRYSIN AND VACCINE.	4	5/10 23	2/12 25	26	15 $	25/2 25	+	O O + +						D.* Vaccine accident	O
"	11	15/12 23	3/9 28	53	16	25/2 25	+	O +	+ +	O O	+			D.* Peritonitis after vaccine.	O
"	12	20/12 23	17/3 24	65	16 $	25/2 25	+	+ +	+ +	+ +	+ +			K. Poor milker.	O
"	44	19/2 25	6 27	32	$ 2 5	5/5 25		O + +	+ +	+ +				K.* Sterile. Fresh & calc. Tb	+
SANOCRYSIN	6	1/11 23	27/2 28	52	14		+	+ + +	+	+ +				S. Sterile.	
"	7	18/11 23	6/9 27	38	13		+	+ + +	+	+				S. Sterile.	
"	10	1/12 23	18/12 28	61	12		+	+ + +	+	+ +	+ +			S. Sterile.	
"	16	23/2 24	13/9 27	42	11		+	+ + +	+	+ +				K. Sterile. Fresh Tb.	+
"	18	1/3 24	29/1 27	31	10		+	+ + +	+	+				K. Fresh Tb.	+

$ Sanocrysin treatment from 24/6/25 to 22/7/25.

TABLE 4
CONTROLS.

EXP. No.	BORN	OUT DATE	OUT AGE MONTHS	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	REMARKS. S.= Sold. K.= Killed. D.= Died. * = Details in Post Mortem Notes.	Post Mortem TB Lesions O or +
2	27/3 23	11/9 33	115	O	+	+ +	+ + +	+ +	+	+	+	+	+	S. Sterile	
3	1/9 23	23/9 29	67	O	+	+ +	+ + +							K. Poor Milker; Fresh TB.	+
5	30/10 23			O O	O O + +	+ +	+ + +	+ +	+	+	+	+	+		
9	25/11 23	4 26	29	O	O									S. Foot & Mouth Disease.	
14	23/7 24			O	+	+ +	+ + +	+ +	+	+	+	+	+		
15	5/3 24	10 29	67	O O	+ + + +	+ +	+ + +							K. Sterile. Fresh TB.	+
17	23/3 24	4 27	36	O	+ + +									S. Sterile.	
19	1/4 24	3 27	31	O O	O O O + +									K. Sterile. Much fresh TB.	+
24	29/4 24	11/5 27	38	O O	O O O + + +									S. Abortion.	
30	16/6 24	19/3 27	29	O	O O O + +									D.* Tympanitis. Fresh TB.	+
34	25/6 24	9/5 26	22	O	O + + +									K.* Fresh TB.	+
47	5/3 25	30/9 26	18		O O O O + +									K.* Fresh TB.	+
53	17/4 25	4 26	12		+									D. Foot & Mouth Disease.	
321	23/10 32	21/11 32	1									+		K.* Fresh TB.	+

TABLE V.

ANIMALS BOUGHT IN - IMMUNISED.

EXP. No.	BORN	BOUGHT DATE	BOUGHT AGE MONTHS	OUT DATE	OUT AGE MONTHS	OUT MONTHS IN HERD	DATE FIRST DOSE VACCINE	TUBERCULIN TESTS. 1927	1928	1929	1930	1931	1932	1933	REMARKS. S.= Sold. K.= Killed. D.= Died. * = Details in Post Mortem Notes.	Post Mortem TB Lesions O or +
♂109	4/5 25	23/11 26	14	1/12 30	63	44	28/10 26	+	+ +	+ +					K.* Sterile.	+
130	♀ 25	? 27	19	15/5 32	83	64	25/10 27		+ +	+ +	+ +	+ +	+		S. Abortion.	
131	♀ 25	? 27	19	23/3 31	69	50	22/11 27		O + +	+ +	+ +				S. Mastitis.	
132	♀ 25	? 27	19				22/11 27		O + +	+ +	+	+ +	O	O +		
133	♀ 25	? 27	19				5/11 27		O + +	+ +		+ +	O	+ O +		
134	2/12 26	16/10 27	10	25/2 29	27	17	25/4 28	+	+ +						K.* Fresh Tuberculosis.	+
142A	♀ 24	13/10 27	40	23/2 33	104	64	22/11 27		+ +	+ +	+ +	+ +	+ +		S. Mastitis.	
142B	♀ 25	13/10 27	28				22/11 27		+ +	+ +	+ +	+ +	+ +	+		
142C	♀ 25	1/10 27	28				22/11 27		O +	+ +	+ +	+ O	+ +	+ +		
142D	♀ 25	8/10 2,	28	15/10 29	52	24	22/11 27		+ +							
142E	♀ 25	13/10 27	28	30/10 32	88	60	22/11 27		O +	+ +	+ +	+ +	+		S. Sterile.	
142F	♀ 25	9/10 27	28	28/10 29	52	24	22/11 27		+ +	+					S. Sterile.	
142G	♀ 25	8/10 27	28	12/7 31	73	45	22/11 27		+ +	+ +		+	O		K.*	O
♂142H	23/6 26	25/9 27	15	28/4 32	70	55	22/11 27		+ +	+ +	+	+ +			S. Sterile.	
142I	♀ 25	8/10 27	28	5/5 30	59	31	22/11 27		O +	+ +	+				S. Vicious.	
142K	♀ 25	23/10 27	28	18/12 28	42	14	22/11 27		+						S. Sterile.	
142L	♀ 25	8/10 27	28				22/11 27		+ +	+ +	+ +	+ +	+ +	O	S. Mastitis.	
142M	♀ 25	8/10 27	28	8/4 29	46	18	22/11 27		+							
♂209	♂ 29	♂ 29	5				20/11 29			O	+ +	+ +	+ +	O	S. Sterile.	
210	♂ 27	♂ 29	26	1/2 31	73	17	20/11 29			O	O O					
211	♂ 28	♂ 29	15	23/2 33	60	45	20/11 29			O	+ +	+ +	+ +		K. Foot & Mouth Disease.	O
212	♂ 27	♂ 29	22				20/11 29			+	+	+ +	+ +	+	K.* Sterile.	O
213	♂ 28	♂ 29	12				20/11 29			O	+ +	+ +	+ O	+		
214	♂ 27	♂ 29	22	1/3 32	56	34	20/11 29			O	O O	O +				
215	♂ 27	♂ 29	22	1/3 31	44	22	20/11 29			O	+ +				S. Sterile.	
216	♂ 27	♂ 29	19				20/11 29			O	O O	+ +	+ +	+	S. Sterile.	
217	♂ 27	♂ 29	22	25/3 31	44	22	20/11 29			O	+ +					
218	♂ 27	♂ 29	22				20/11 29			O	+ +	+ +	+ O	O	K. Mastitis.	O
219	♂ 28	♂ 29	10	17/11 31	31	21	20/11 29			O	C O					
220	♂ 27	♂ 29	22	25/2 32	55	33	20/11 29			O	+ O	O +			S. Sterile.	
															S. Sterile.	

TABLE 6. - VACCINE GROUP.

EXP N°	BORN	OUT DATE	OUT AGE	AGE FIRST DOSE	DOSE	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	REMARKS.	Post Mortem TB lesions 0 or +
8	3/25	5/27	26	375	0	0	+	+	+	+						S. Abortion.	
20	4/24			114	0	0	0	0	+	+	+	+	+	+	+		
21	4/24			114	0	0	0	0	+	+	+	+	+	+	+		
22	4/24	3/27	36	102	0	0	0	+	+	0	+					S. Abortion.	
23	4/24			96	0	0	0	0	+	+	+	+	+	+	+		
25	4/24	5/32	92	96	0	0	0	0	+	+	+	+	0	+	+	S. Poor Milker.	
26	4/24	5/32	93	928	0	0	0	0	+	+	+	+	0	+	0	S. Poor Milker.	
27	4/24	-		71	0	0	0	0	+	+	+	+	+	+	+		
28	4/24	7/28	50	58	0	0	0	+	+	+	+	+	+			S. Poor Milker.	
29	4/24	7/30	71	51	0	0	0	+	+	+	+	+				S. Vaginitis.	
31	4/24	3/27	36	43	0	0	0	0	0	+	0	+	+			S. Sterile.	
33	4/24	3/27	35	12	0	0	0	0	0	0	+	+				S. Sterile.	
35	4/24	5/32	89	13		0	0	+	+	+	+		+	+	+	+	0	+		S. Sterile.	
36	4/24	12/33	100	8		0	0	0	0	+	+	+	+	+	+	+	+	0	0	S. Sterile.	
37	4/24	8/31	84	15		0	0	+	+	+	+	+	+	+	0	0	+			K. Poor Milker.	
39	7/25	7/25	5	23		0	+									K.* Calcified TB.	0
40	8/25	7/26	20	10		0	0	+	+	+	+					K.* Calcified TB.	+
41	4/25			7		0	0	0	0	+	+	+	+	+	+	+	0	+	0		
42	4/25	7/26	22	5		0	0	+	+	+	+					K.* Fresh & Calc. TB.	+
43	1/25	4/26	12	5			0	0	0	0						K. Tympanitis.	0
45	7/25	3/33	98	5			0	0	+	+	+	+	+	+	+	+	+	+	0	D. Vaccine accident.	
46	4/25	3/30	62	11				0	0	0	+	+	+	+	+					S. Abortion.	
48	8/25			24				0	0	0	+	+	+	+	+	+	0	0	0		
49	4/25	8/27	30	1				0	0	+	0					S. Abortion.	
50	1/25			3				0	0	+	0	+	+	+	+	0	0	0	0		
51	6/25			11				0	+	0	+	0	+	+	+	+	0	+	0		
52	4/25			2				0	0	0	+	+	+	+	+	+	0	+	0		
54	4/25	3/26	12	25				0	0							S.	
55	7/25	3/30	64	20				0	0	0	+	+	+	+	+	+				S. Three tests.	
56	7/25	7/29	93	3						0	0	+	+	+						S. Poor Milker.	
57	8/25	3/31	68	2						0	0	+		+	+	+		+	0	S. Sterile.	
58	4/25			1						0	0	+	+	+	+	+	+	+	+		
59	4/25	7/26	64	1						0	0	+	+	+	+	+	+			S. Sterile.	
60	8/25	7/28	28	*						0	0	+	+							D. During calving.	0
61	4/25	7/29	41	*						0	0	+	+	+						K. Sterile.	0
62	6/25	7/28	38	2						0	0	+	+	+	+					S.	
63	3/25	3/26	5	4												D. Fe 1 & Mouth Dis.	0
64	5/25	7/28	37	3						0	0	+	+	+						S. Poor Milker.	
65	8/25			9						0	+	+	+	+	+	+	+	+	+		
66	7/25	3/26	8	3						+						S.	
67	8/25			2						0	0	+	+	+	+	+	+	+	0		
68	8/25	7/28	37	9						0	0	+	+	+	+					S. Sterile.	
69	8/25	3/33	89	7						0	0	+	+	+	+	+	+	+	0	S. Poor Milker.	
70	4/25	4/27	21	2						0	0	+								K.*	0
71	4/25	4/28	35	7						0	0	0	0							K. Sterile	0
72	4/25			2						0	0	+	+	+	+	+	+	+	0		
73	7/26	3/33	80	14						0	0	+	+	+	+	+	+	+	0	S. Sterile.	
74	4/26			9						0	0	+	+	+	+	+	+	+	0		
75	4/26			7						0	0	0	+	+	+	+	0	+	+		
76	3/26			8						0	0	0	+	0	+	+	0	0	+		
77	7/26	7/26	5	3						0						K.*	0
78	4/26			3						0	0	0	+	+	+	+	+	0	+	0	
79	3/26			2						+	+	0	+	+	+	+	0	+	+	0	
80	4/26			2						0	0	0	+	+	+	+	+	+	+	0	
81	4/26			2						0	0	0	+	+	+	+	+	+	0	0	
84	3/26			3						0	0	0	+	+	+	0	0	+	+	0	
85	4/26			1						0	0	0	+	+	+	+	+	0	+	0	
86	4/26	4/29	34	4						0	+	+	+							S. Abortion.	
87	4/26			2						0	0	0	+	+	+	+	0	+	0	0	0
88	4/26	4/27	11	3							0					S.	
90	4/26	3/31	61	1							0	0	+	+	+	+	+	+		S. Sterile	
91	4/26	4/28	30	4							0	0	0	0	+					S. Abortion	
92	4/26	4/28	30	4							0	0	0	+	+					S. Sterile.	
93	4/26	4/29	29	4							0	0	0	+						S. Abortion.	
94	"	"	41	13							0	0	+	+	+					K. Abortion, Nephritis	0
95	4/26			10							0	0	+	+	+	+	0	+	+	+	+
96	4/26	8/30	46	3							0	+	+	+	+	+				S. Broken leg.	
97	4/26			3							0	+	+	+	+	+	+	0	+	+	+
98	4/26	5/30	51	1							0	+	+	+	+	+				S. Abortion.	
99	4/26	4/30	48	1							0	+	+	+	+	+				K. Strep. abscesses.	0
100	4/26	5/30	49	4								0	+	+	+	+				S. Abortion.	
101	4/26			2								+	+	+	+	+	+	+	+	+	+
102	4/26	4/30	41	11								+	+	+	+	+				K. Sterile	0
104	4/26	4/28	38	9								+	+	+	+	+				S. Sterile.	
105	4/26			8								0	0	+	+	0	+	+	+	+	0
106	4/26	7/30	42	6								0	+	+	+	+	+			S. Sterile.	
107	4/26			6								0	+	+	+	+	+	0	+	0	0
108	4/26	4/31	5	3								0	+	+	+	+	+	+	+		
110	4/26			7								0	0	+	+	+	+	+	+	0	+
111	4/26			7								0	+	+	+	+	+	+	+	0	0
112	4/26	4/30	38	4									+	+	+	+				K. Virulous.	0
113	4/26	4/29	31	1									+	+	+	+				S. Metritis	0
114	4/26	4/28	28	2									0	+	+	+				S. Sterile.	
115	4/26	4/29	29	2									0	0	+					S. Abortion.	
116	4/26			4									0	0	+	+	+	+	+	+	+
117	4/27	4/31	51	4										+	+	+	+	+		S. Sterile.	
119	4/27	4/30	41	8										0	+	+	+	+		K. Sterile.	0
114	4/27	4/28	44	8										0	+					D. Tympanitis.	0
120	4/27			7										0	+	+	+	+	+	+	0
121	4/27			5										0	+	+	+	+	0	+	0
122	4/27	4/30	35	7										0	0	+	+	+		S. Abortion.	
123	4/27			7										0	+	+	+	+	+	+	+
124	4/27			3										+	+	+	+	+	+	0	0
125	4/27	4/28	18	4										0	0					D. Tympanitis.	0
126	4/27	4/28	19	10										0	+					K.*	0
127	4/27	5/30	71	2										0	+	+	+	0	0	+	+
128	4/27	4/29	25	6										0	+	+	+			K. Difficult calving.	0
129	4/27			4										0	+		+	+	+	+	0
135	4/27	4/28	11	1										0	+					D. Tympanitis.	0
136	4/27			1											0	+	+	+	+	+	+

TABLE 6 cont'd
VACCINE GROUP.

EXP. No.	BORN	OUT DATE	OUT AGE	AGE FIRST DOSE MONTHS	AGE FIRST DOSE DAYS	TUBERCULIN TESTS 1930	1931	1932	1933	REMARKS. S.= Sold. K.= Killed. D.= Died. *= Details in Post Mortem Notes.	Post Mortem TB lesions 0 or +
137	1/27	1/28	13	10	0						
138	2/27			12	+	+	+	+	+		
139	2/27	8/31	46	6	+	+	+	+	+		
140	2/27	4/28	8	4	0						
141	2/27			7	0	0	+	+	+	+	0
142	2/27			5	0	+	+	+	+	0	0
143	3/27	8/28	24	7	+	+	+				
144	3/27			2	0	+	+	+	+	+	0
145	3/27			19	+	+	+	+	+	0	0
146	3/27	12/28	10	5	+	+					
147	4/27			3	0	+	+	0	+	+	+
148	4/28			12	+		+	+	+	+	+
149	4/28			5		+	+	+	+	+	+
150	4/28			33		+	+	+	+	+	0
151	4/28										
152	4/28	4/28	6	3						E. Pleuropneumonia.	0
153	4/28	4/28	6	24						E. Pleuropneumonia.	0
155	4/28			1		0	0	+	+	+	0
156	4/28			20		+	+	+	+	+	+
157	4/28			12		0	0	+	+	+	0
158	4/28			8		0	0	+	+	+	+
159	7/26	4/33	58	13		0	0	+	+	+	+
160	7/28			13		+	0	+	+	+	+
162	5/28	8/31	39	11		0	0	+	+	+	
163	4/28			7		+	+	0	+	+	+
164	4/28			6		+	+	0	0	+	0
165	4/28			3		0	0	+	+	+	+
166	5/28			3		+	0	+	+	+	+
167	5/28			4		0	+	0	0	+	+
168	5/28			3		0	0	0	+	+	0
169	5/28			2		0	0	0	+	+	+
170	5/28	4/31	35	1		0	+	0	0	D.* Accident.	0
171	5/28			5		0	+	0	0	+	+
172	5/28			3		0	0	0	+	0	+
173	5/28	8/31	26	9		0	0	0	0	S. Abortion.	
174	5/28			26		0	0	0	0	+	0
175	5/28			20		0	0	0	+	+	+
176	5/28			20		0	0	+	+	+	+
177	5/28			13		0	0	0	+	+	+
178	6/28	8/32	36	4		0	0	0	+	S. Sterile.	
179	6/28	8/32	46	15		0	0	+	+	+	0
180	6/28	8/32	48	113		0	+	+	+	+	0
181	6/28	8/33	52	69		0	0	0	0	S. Sterile.	
182	6/28			141		+	0	0	0	+	+
183	6/28	8/32	42	25			0	0	0	+	+
185	6/28			12			0	0	+	+	+
186	6/28			34			0	+	+	+	+
187	8/28	8/29	3	5		0				D. Foot & Mouth Dis.	0
188	8/28			3			0	0	0	0	+
189	8/28			12			0	0	0	+	+
191	8/28	8/32	38	7			0	0	0	+	+
192	8/28	8/32	36	5			0	0	0	+	0
193	8/28			5			0	0	0	+	+
195	8/28			7			0	0	0	+	+
196	8/28			12			0	0	0	+	+
197	8/28	8/33	46	11			0	+	0	+	+
198	8/28	8/33	45	8			0	+	0	0	+
199	8/28			29			0	0	+	+	+
200	8/28			8				0	0	+	+
201	8/28			23				0	0	+	0
202	8/28	8/32	38	8				0	0	+	+
203	8/28			8				0	0	+	+
204	8/28	8/30	8	8				0	0	+	+
205	8/28			11				0	0	+	0
206	8/28			25				0	0	+	0
207	8/28			24				0	0	+	+
208	8/28			24				0	0	0	+
221	8/28			20				0	0	+	+
222	8/28			11				0	0	+	+
223	8/28	1/30	3	4						K.*	0
224	8/28			23				0	0	+	+
225	8/28	4/32	27	18				0	+		
226	8/28			15				0	+	0	0
227	8/28			6				+	+	0	+
228	8/28			31				+	+	+	+
230	9/28	8/31	12	26				+		S.	
231	9/28	8/32	27	24				+	+	+	
232	8/28			21				0	+	+	+
234	8/28	8/32	22	19				0	+	0	
235	8/28			19				+	+	0	+
236	8/28	8/31	17	4				+	+		
237	8/28			27				+	0	+	+
238	8/28	8/32	29	15				+	0	0	
239	8/28	8/32	28	10				+	+	+	
240	8/28	8/33	32	7				+	0	0	
241	8/28	8/33	33	44				0	+	+	
242	8/28			42				+	+	0	0
243	8/28			42				0	+	+	0
244	8/28	8/33	35	5				+	+	+	0
245	8/28			22				0	0	+	+
246	8/28	8/33	31	21				0	+	+	+
247	8/28			8				0	+	+	+
248	8/28			5				0	+	+	+
249	8/28	8/33	30	17				0	+	+	+
250	8/28			9				0	+	+	+
251	8/28			4				0	0	+	+
252	8/28	8/31	4	59						D.* Vaccine accident.	0
253	8/28			17					0	0	0
254	8/28			9					0	0	+
255	8/28			38					0	+	+
256	8/28			16					0	0	+

TABLE 6 cont'd
VACCINE GROUP.

EXP. No.	BORN	OUT DATE	OUT AGE	AGE FIRST DOSE MONTHS	AGE FIRST DOSE DAYS	TUBERCULIN TESTS 1931	1932	1933	REMARKS. S.= Sold. K.= Killed. D.= Died. *= Details in Post Mortem Notes.	Post Mortem TB lesions 0 or +
257	8/31			7	0	0	0	+		
258	8/31			4	0	0	0	0		
259	8/30			117	0	+	+	+		
260	8/31			27	+	+	+	+		
261	8/31			27	+	+	+	+		
262	8/31			12	+	+	+	+		
264	8/31			4	0	0	0	+		
265	8/31			3	0	0	0	+		
266	8/31			1	0	0	0	+		
267	8/31			61	0	0	0	+		
268	8/31			46	0	0	0	+		
269	8/31			18	0	0	0	+		
270	8/31			15	0	0	0	0		
271	8/31			13	0	0	0	0		
272	8/31			21	0	0	0	0		
273	8/31			30	0	0	0	+		
274	8/31			30	0	0		0		
275	8/31			10		0	0	+		
276	8/31			4		0	0	0		
277	8/31			29		0	0	0		
278	8/31			27		0	0	+		
279	8/31			21		0	0	+		
280	8/31			20		0	0	0		
281	8/31			19		0	0	0		
282	8/31			11		0	0	+		
283	8/31			4		0	0	0		
284	8/31			16		0	0	+		
285	8/31			12		0	0	0		
286	8/31			10		0	0	0		
287	8/31			6		0	0	0		
288	8/31			1		0	0	0		
289	8/31			26		0	0	0		
290	8/31			25		0	0	0		
291	8/31			21		0	0	0		
292	8/32			6		0	0	0		
293	8/32			23		0	0	0		
294	8/32			21		0	0	0		
295	8/32	8/32	10	6		0	0		D. Constipation.	0
296	8/32			6		0	0	0		
297	8/32			11		0	0	0		
298	8/32			2		0	0	0		
299	8/32			9		0	0	0		
300	8/32			7		0	0	0		
302	8/32			29			0	0		
303	8/32			25			0	0		
304	8/32			34			0	0		
305	8/32	8/33	10	24			0		K. Too small.	0
306	8/32			18			0	0		
307	8/32	8/32	3	9					D.*Vaccine accident	0
308	8/32			29			0	0		
309	8/32			29			0	0		
310	8/32			28			0	0		
311	8/32			19			0	0		
312	8/32			18			0	0		
313	8/32			18			0	0		
314	8/32			10			0	0		
315	8/32	8/33	7	7			0	0	D.* Cholecystitis.	0
316	8/32	8/32	3	4			0		D. Bronchopneumonia	0
317	8/32	8/33	5	43			0		D. Enteritis	0
318	8/32			31				0	0	
319	8/32			28				0	0	
320	8/32			26				0	0	
322	8/32			22				0	0	
323	8/32			21					0	
324	8/32			17					0	
325	8/32			8					0	
326	8/32			30					0	
328	8/32			13					0	
329	8/32			4					0	
330	8/32			18					0	
331	8/33			6					0	
332	8/33			6					0	
333	8/33			17					0	
334	8/33			15					0	
335	8/33			15					0	
336	8/33			12					0	
337	8/33			8					0	
338	8/33	8/33	3	7					D. Enteritis.	0
339	8/33			5					0	
340	8/33			2					0	
341	8/33			10					0	

TABLE 7.

The Number of Animals which have been in the Herd for Periods of 3 to 8 Years, and the Number of these which have been Tuberculin Negative after having been Positive.

Months in Herd	Number of Animals	Number O after +	Per cent O after +
36	134	72	53.7
48	90	60	66.7
60	61	48	78.7
72	43	38	88.4
84	30	28	93.3
96	8	8	100.0

TABLE 8

VACCINE GROUP: TUBERCULIN REACTIONS OF DIFFERENT AGE GROUPS.

AGE GROUP. MONTHS.	1924 30/5 - 2/6		1925 31/5-2/6 1/6-6/6		11/5-1926		6/5-1926		16/4-1927		16/10-1927		18/4-1928		27/11-1928		22/5-1929		7/11-1929		18/5-1930		12/11-1930		22/4-1931		27/10-1931		12/4-1932		16/11-1932		25/5-1933		1924 - 1933 ALL TESTS.				AGE GROUP MONTHS.
	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	T	O	TOTAL	0	+	% O	
1 - 6	16	0	40	3	26	2	25	2	12	6	1	0	11	6	-	-	22	3	5	0	12	0	12	5	9	0	12	1	27	0	18	0	30	0	268	242	26	90.3	1 - 6
7 - 12	2	0	39	11	4	0	19	0	14	6	24	10	2	0	6	5	19	4	9	2	9	2	10	7	13	12	11	5	27	0	26	0	7	0	234	167	67	71.4	7 - 12
13 - 18			11	4	13	9	7	1	22	13	14	9	24	22	3	3	10	9	15	4	25	5	9	0	17	8	13	9	9	5	12	0	26	5	230	123	107	53.5	13 - 18
19 - 24			2	2	10	9	16	13	7	7	21	14	24	13	24	14	1	1	8	7	8	8	17	4	7	3	17	13	16	12	12	8	11	9	181	44	137	24.3	19 - 24
25 - 36					1	1	3	3	19	19	22	12	26	25	35	32	30	30	18	18	12	12	25	18	33	21	27	24	21	17	21	19	20	15	303	37	266	12.2	25 - 36
37 - 48									1	1	2	2	16	16	25	15	18	17	27	26	24	24	33	13	11	11	22	20	29	24	22	21	17	12	217	15	202	6.9	37 - 48
49 - 60															7	7	13	13	33	12	17	16	22	15	15	14	12	10	17	10	23	16	26	23	160	24	136	15.0	49 - 60
61 - 72																			7	7	13	11	21	10	9	1	18	12	13	10	11	10	10	7	101	16	85	15.8	61 - 72
73 - 84																							6	6	0	0	14	12	16	8	20	14	15	6	83	29	54	34.9	73 - 84
85 - 96																													11	7	8	4	17	3	42	22	20	52.4	85 - 96
97 -103																															4	0	7	2	11	9	2	81.8	97 -103
1 -103	18	1	92	29	54	22	80	19	75	50	74	47	93	82	78	70	102	77	113	70	130	78	133	78	137	94	108	102	108	98	179	92	186	82	1830	728	1102	39.8	1 -103

TABLE 9.

Tuberculin Tests of No. 127.

Date of Test.	Measurement of Skin Fold			Result of Test	Vaccine in Intervals of Tests c.cms.
	Before Injection	48 Hours	72 Hours		
16.10.27	5	5	5	0	15
18. 4.28	7	15	14	+	15
27.11.28	7	24	25	+	21
7.11.29	8	11	11	+	75
18. 3.30	6	12	11	+	15
12.11.30	6	11	9	+	40
22. 4.31	7	9	9	0	0
27.10.31	8	11	10	0	15
12. 4.32	7	10	10	0	30
16.11.32	6	15	11	+	20

THE RESULTS OF THE DOUBLE INTRADERMAL TEST FOR TUBERCULOSIS IN A SMALL DAIRY HERD.

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This report contains a record of the results of the double intradermal test for tuberculosis applied to a small dairy herd producing Grade A (tuberculin-tested) milk. Recently these results have been disastrous to the herd, because in 18 months 14 animals, in a herd which at its maximum contained 29 cows, failed to pass the test, and therefore had to be removed from the herd.

Since October, 1927, the herd has been licensed to sell Grade A (tuberculin-tested) milk. The first tuberculin test was carried out in October, 1927, the subcutaneous and ophthalmic methods of testing being used, and out of a total of 24 cows, eight gave positive reactions. At all subsequent tests the double intradermal method of testing was employed, and in June, 1930, we became responsible for the tests and for veterinary advice when that was required. The second herd test was carried out in June, 1928, and thereafter, until the end of 1932, the tests were made at six-monthly intervals. Two cows out of 18 reacted at the second test, and four out of 23 reacted at the third test. The fourth and fifth tests showed no reactors. The sixth and seventh tests gave one reactor each, the number of cows tested being 19 and 22 respectively. Both of the cows reacting at the sixth and seventh tests had been purchased as tuberculin-tested cows, the one about six months and the other about twelve months before reacting. After the sixth herd test, which was carried out in June, 1930, the purchase of cows to replenish the herd was stopped, and from that date the only animals purchased for the recruitment of the herd were unserved heifers. Such animals were tuberculin-tested, and the non-reacting animals were maintained in strict isolation until they were due to calve or had calved, when they were again tuberculin-tested before entering the herd; similar precautions to guard against the introduction of infection were taken with the home-bred heifers. Furthermore, in an endeavour to prevent the spread of any possible infection, the herd since April, 1930, has been kept out of doors, only entering the shed to be milked and fed. At the eighth, ninth and tenth tests, covering a period from June, 1931, to June, 1932, no positive reactions occurred.

At the eleventh test, held in December, 1932, three animals gave a positive reaction and the remainder of the herd, 26 in number, and also two heifers shortly due with their first calf, were returned as non-reactors. The status of the non-reactors was queried by the Ministry of Health because of the skin measurements recorded by three of the animals, and instructions to retest

the non-reacting animals were received. At the retest (the twelfth test) held in January, 1933, the three cows whose reactions called forth the retest passed, but one animal, negative to the test in December, reacted, and five others gave reactions which were classified as doubtful. Prior to the twelfth test the Ministry of Health recommended that the tests should be carried out according to the criteria given in the Medical Research Council's Special Report Series, No. 94, and that a tuberculin of known high potency such as that supplied by the Institute of Animal Pathology, Cambridge, should be used. We were not unfamiliar with the Report mentioned. At the twelfth herd test and all subsequent tests the tuberculins used were supplied by the Institute of Animal Pathology, Cambridge.

Following upon the results of the twelfth test the Ministry of Health decided that they should be responsible for another retest of all the alleged negative and doubtful reactors, and in March the thirteenth herd test was made by them. Twenty-five cows were tested; 21 of these had given negative reactions and four had given doubtful reactions in January; the fifth animal giving a doubtful reaction in January had, in the meantime, been removed from the herd, Johne's disease having been suspected. As a result of the March test, six cows were classified as positive reactors; only one of these had given any reaction in January and that a doubtful one, the remainder having been consistently negative at all previous tests since entering the herd.

The remainder of the depleted herd, augmented by the addition of fresh heifers, was tested last October and at this fourteenth herd test, four cows out of a total of 23 gave positive reactions.

It follows, therefore, that in the period from December, 1932, to October, 1933, out of a dairy herd which at its maximum numbered 29 cows, 14 had to be removed because of positive reactions to the double intradermal test and another five gave doubtful reactions to the test during this same period.

TABLE I.

TABLE SHOWING THE RESULTS OF HERD TESTS.

Test.	*Year.*	*Cows Tested.*	*Reacted.*
2nd	June, 1928	18	2
3rd	December, 1928	23	4
4th	June, 1929	20	0
5th	December, 1929	21	0
6th	June, 1930	19	1
7th	December, 1930	22	1
8th	June, 1931	22	0
9th	December, 1931	30	0
10th	June, 1932	27	0
11th	December, 1932	29	3
12th	January, 1933	27	1*
13th	March, 1933	25	6
14th	October, 1933	23	4

*Five doubtful reactors.

In seeking an explanation for these occurrences the following hypotheses present themselves :—

(1) Accepting the double intradermal test as reliable and a positive reaction to be a true index of tuberculosis infection, the veterinary surgeons interpreting the sixth to the thirteenth tests inclusive were incapable of applying the test correctly. They were therefore missing the infected animals, and this culminated in the high percentage of presumably tuberculous animals detected at the thirteenth and again later at the fourteenth test. The possibility of such a view is evident because the Ministry of Health appeared to have suspected the reliability of the tests and accordingly recommended the study of a Report devoted to the double intradermal test and the use of a particular tuberculin; and furthermore, as no interest was taken in the past or future histories of the six animals which reacted to the test conducted by them in March, it is to be assumed that they were regarded by the Ministry of Health as cases of tuberculosis and that a comparatively heavily infected herd existed even after six years of repeated testing. The gravity of such a state of affairs needs no comment. Nevertheless, we are unaware of its having excited any enquiry on the part of the Ministry of Health. It is to be hoped that it was merely an isolated instance inadvertently overlooked.

Again the classification of animals as doubtful reactors at the thirteenth test may well have been attributed to incompetence in the execution of the tests, because Glover (1930), in the capacity of an authority on the interpretation of the test, said, regarding the differential characteristics of the swellings in positive and negative cows, " In spite of these distinctions, experience in the field has convinced us that many veterinary practitioners are still unable to draw distinction between reactor and non-reactor," and later, in a letter in *The Veterinary Record*, Glover (1930) stressed the point that a veterinary surgeon, by his recognition of the doubtful reactor, had not yet mastered the interpretation of the double intradermal reaction, and said " Past experience has clearly shown that such cases are often misinterpreted and should be regarded as reactors." When our results are considered in the light of such authoritative statements there can be little wonder if the validity of the tests was questioned.

(2) The introduction of infection from without.

This hypothesis is unlikely because of the methods used in recruiting the herd since 1930, unless of course the testing of the animals introduced had been faulty.

(3) Infection from other classes of stock or from human beings.

The only other animals occasionally in contact with the herd were sheep. No suspicion of tuberculosis among any of the herd attendants was entertained.

(4) Unreliable tuberculin used prior to January, 1933.

Later it will be seen that anomalous results were obtained

with the tuberculin used after December, 1932. Furthermore, there is no cause to suspect that cases of tuberculosis have been missed because a poor quality of tuberculin may have been used prior to January, 1933.

(5) Non-specific sensitisation to tuberculin.

Jordan (1933), in discussing the anomalous results in a herd where 18 animals reacted to the double intradermal test and six months later 14 of the same animals passed the test, states that just prior to the first of these tests the animals had been vaccinated against contagious abortion, and that five of the animals developed suppurative swellings. Particulars of the vaccine used are not given, but it is probable that massive inoculations of a broth culture of *Br. abortus* were given. Should the vaccination have been responsible for non-specific reactions to tuberculin these may have been induced by : (1) The inoculation of large quantities of broth sensitising the animals to the broth in the tuberculin. (2) A non-specific sensitisation caused by *Br. abortus*. (3) A non-specific sensitisation caused by the suppurative process which followed vaccination.

A number of the animals in the herd we are considering had been vaccinated on several occasions with small doses, 2 c.c. of a saline suspension of *Br. abortus,* obtained by washing off 48 hour agar slant growths of *Br. abortus*. These suspensions were relatively dilute, and any derivatives from the broth used to make the culture media must have been present in minute traces only. Therefore sensitisation caused by the inoculation of such derivatives is unlikely. This is borne out by the fact that approximately half of the animals which gave positive or doubtful reactions to the tuberculin test had never been vaccinated, which also makes the suggestion that non-specific reactions could be due to the inoculation of *Br. abortus* untenable. Finally, no suppurative or other swellings occurred in the vaccinated animals.

A sensitisation to tuberculin caused by the repeated inoculations of tuberculin or johnin is unlikely, as positive reactions occurred in December, 1932, before the period between tests had been reduced to less than six months. Furthermore, some animals giving positive reactions when tested only a short time later gave negative reaction.

(6) That positive reactions to the double intradermal test had occurred which were independent of any specific sensitisation of the animal to any material in the tuberculin.

Retests and Post-mortem Examinations of Animals Discharged from the Herd as Reactors to the double Intradermal Test.

The animals showing positive reactions at the eleventh, twelfth and thirteenth herd tests were isolated from the herd but were then running in the same field and using a common yard with three reacting animals which had never been in the dairy herd.

In April, 1933, a retest of the reacting cows from the herd was made. The interpretation of the test is given below :—

Name.	*Date when Animal gave a Positive Reaction.*	*Result of April Test.*
Cherry	December	Positive
Red Rose 29th	„	„
Bessie	„	Negative
Missie	January	Positive
Mina	March	Negative
Bess	„	„
Ruth III	„	„
Kathleen II	„	„
Violet	„	Positive
Lena	„	„

These results showed that there was no constant correlation between the results of the two tests and they corresponded to the anomalous results previously obtained when animals giving doubtful reactions had been retested.

All five of the animals giving negative reactions in April were slaughtered and no microscopic lesions of tuberculosis were found at the *post-mortem* examinations. Material from the lymph glands from three of these animals was inoculated into guinea-pigs at this laboratory and the guinea-pigs when killed two-and-a-half to three months later were free from tuberculosis.

Further, an animal giving a positive reaction in March and again in April was slaughtered, but no tuberculosis lesions were found at the *post-mortem* examination, and guinea-pigs inoculated with material from the lymph glands were free from the disease when killed and examined three months later. The Ministry of Health was represented at the *post-mortem* examinations on the cows.

Material from the lymph glands of all six cows was inoculated into guinea-pigs at the Institute of Animal Pathology, Cambridge. Six months later a report was received stating that none of the guinea-pigs had shown evidence of tuberculosis when killed and examined. The evidence therefore shows that none of the six cows which were slaughtered were affected with tuberculosis.

In October, the four remaining cows, Cherry, Red Rose 29th, Missie and Lena, were tested by the subcutaneous and the intradermal methods. Three of the cows passed the subcutaneous test, the fourth cow, Missie, calved during the course of the test thereby rendering the test inaccurate, but as far as could be judged, her reaction was negative. All four cows passed the intradermal test. At the same time the three reacting animals with which these cows had been running were also tested. Two gave a positive reaction to the intradermal test and one gave a doubtful reaction; one gave a positive reaction to the subcutaneous test, another

showed a thermal reaction which was suspicious, while the third was negative to the subcutaneous test.

In December, 1933, the cow Missie was found ill and unable to rise. It was reported that the animal had been ill for one or two days and, as recovery appeared improbable, she was slaughtered and a *post-mortem* examination made. No lesion of tuberculosis was found and guinea-pigs inoculated with emulsions of the lymph glands showed no evidence of disease when killed two months later.

The four cows which reacted to the fourteenth herd test in October were retested within 24 hours of the completion of the first test and all passed the test. The tuberculin used at both tests came out of the same bottle. The syringes and needles were always sterilised by boiling before use. The Ministry of Health was informed of the results of the retest but ruled that the animals had to be removed from the herd. These animals were, therefore, isolated, and four weeks later tested by the subcutaneous test and the double intradermal test which was carried out in duplicate, using both the ordinary and a " synthetic " tuberculin, the latter kindly supplied by Professor Buxton. The four cows passed all these tests.

The negative test following close upon the positive test was not accepted by the Ministry of Health as evidence of freedom from tuberculosis. Upon the work recorded in the Medical Research Council's Special Report Series, No. 94, which forms the foundation for the system of testing accepted by the Ministry of Health to the exclusion of all other methods of tuberculin testing, such an attitude would be difficult to defend. In this Report which was issued by the Tuberculin Committee of the Medical Research Council, the evidence of the reliability of the intradermal test was primarily based upon the results of the test in 114 animals which were slaughtered after completion of the test and examined for tuberculosis. The presence of tuberculosis in certain of these animals was determined by finding microscopic lesions, by demonstrating acid fast bacilli in these lesions, and by the development of tuberculosis in guinea-pigs inoculated with emulsions of lymph glands, material from one bovine being inoculated into each of two guinea-pigs. The absence of tuberculosis in other animals was determined by a failure to find microscopic lesions and the absence of evidence of tuberculosis in two guinea-pigs inoculated with an emulsion of lymph glands from each bovine animal examined.

The evidence so accumulated was very favourable to the test; 39 of the 114 animals reacted to the test and 37 of these, or 94·9 per cent. were found to be tuberculous. Seventy-five animals failed to give a reaction to the intradermal test and only one of these was found infected with tuberculosis. Therefore 98·7 per cent. of animals failing to react to the test were free from tuberculosis.

The Report showed that a negative reaction is just as reliable an indication of the absence of infection as a positive reaction is of the presence of infection.

Therefore, on the grounds of such evidence, there is no reason for refusing to accept a negative reaction as indicating freedom from tuberculosis even when it follows a positive reaction.

The Report further gives the results of tuberculin tests on seven different herds, five of which were heavily infected with tuberculosis. Some of these tests were comparative in nature, being designed to test the relative merits of the subcutaneous, the intradermal and the ophthalmic methods of testing. The conclusions drawn from these tests were that the intradermal test appeared to be superior to the subcutaneous and that the ophthalmic test must be regarded as a subsidiary test. These conclusions regarding the relative merits of the different tests were based upon the reactions given to the tests only, and were not checked up by *post-mortem* examinations except in the case of four animals, which animals came from a herd which had apparently been freed from tuberculosis by using the subcutaneous test. However, at a subcutaneous test on some of the younger animals in this herd, these four had shown thermal reactions and at subsequent retests they behaved in a somewhat anomalous fashion, and at one time one of the animals gave a suspicious reaction to the intradermal test but later passed this test. The four animals were slaughtered and the veterinary surgeon conducting the *post-mortem* examinations reported the presence of tuberculosis lesions in certain glands of two of the animals, and one of these animals was the one which had formerly given a suspicious reaction to the intradermal test. However, there is considerable uncertainty regarding the accuracy of the diagnosis as the lesions were not examined for bacilli and no material was inoculated into guinea-pigs.

The only other regularly tuberculin-tested herd which came within the sphere of the Committee's enquiry consisted of 70 animals, and this herd was brought to the Committee's notice because the veterinary surgeon in charge of the herd had obtained unexpected positive reactions in 16 animals tested by the ophthalmic test. Fourteen of these 16 animals and two others were tested by the Committee, using the intradermal and the ophthalmic tests. Three of the 16 gave a reaction to the ophthalmic test and of these one gave a reaction to the intradermal test, while another gave a "slight but highly suspicious" reaction, and with the third the result of the intradermal test was negative. No *post-mortem* examination was made on any of these animals.

On account of the anomalous results the ophthalmic test was regarded as of subsidiary importance.

The records now submitted show that in a small herd 14 animals

reacted to the double intradermal test between December, 1932, and October, 1933, but that there is no evidence that any of these animals was tuberculous because: (1) Thirteen of these animals subsequently passed the double intradermal test, and the fourteenth after giving two consecutive positive reactions to the test was found free from tuberculosis at *post-mortem* examination. (2) Eight animals which a short time previously had reacted to the double intradermal test passed the subcutaneous test. (3) *Post-mortem* examinations were made on seven of the 14 animals and in no instance was tuberculosis found; two of these seven animals had given positive reactions to the double intradermal test on two consecutive occasions. Therefore, according to the criteria accepted by the Report, the records now submitted regarding the double intradermal test justify its relegation to the level of a subsidiary test.

Appendix.

Post-Mortem Examinations.

Cow Ruth III.

Pregnant.

Age, six years.

History. Negative in seven tests, positive in March, 1933, and negative when retested in April, 1933.

Slaughtered and examined on May 6th, 1933.

Post-mortem Examination.—The animal was in very good condition and no lesions could be found.

Lymph glands were inoculated into guinea-pigs at the Institute of Animal Pathology, Cambridge, and negative results obtained.

Cow Kathleen II.

Pregnant.

Age, six years.

History. Negative in six tests, positive in March, 1933, test and negative when retested in April, 1933.

Slaughtered and examined on May 6th, 1933.

Post-mortem Examination.—The animal was in good condition and the only lesions noted were a few fibrinous adhesions of the pleura and a small pale area in one internal iliac lymph gland. The latter were suggestive of an old pyæmic infection. No lesions of tuberculosis were found.

Lymph glands were inoculated into guinea-pigs at the Institute of Animal Pathology, Cambridge, and negative results obtained.

Cow Bess.

Age, ten years.

History. Negative in ten tests, positive in March, 1933, and negative when retested in April, 1933.

Slaughtered and examined on May 13th, 1933.

Post-mortem Examination.—A small abscess was found in the wall of the reticulum and the peritoneal surface of the organ at this point was roughened, with traces of an inflammatory reaction. It was assumed to be traumatic in origin. A few fibrinous adhesions were

Table II.

Table Summarising the Results of Tuberculin and Other Tests on Animals which gave Positive or Douubtful Reactions to the Tuberculin Test from December, 1932, to October, 1933.

Name of Cow	No. of Tuberculin Tests Passed prior to Dec., 1932.	Intradermal Tests. Herd Test, Dec., 1932.	Herd Test, Jan., 1933.	Herd Test, Mar., 1933.	Special Test, April, 1933.	Herd Test, Oct., 1933.	Retest of Oct., 1933.	Subcutaneous Test. Oct., 1933.	Intradermal Tests. Special Test, Oct., 1933.	Special Test, Nov., 1933. Ordinary Tuberculin.	Special Test, Nov., 1933. Synthetic Tuberculin.	Subcutaneous Test. Dec., 1933.	Post-mortem Examination of Cows. Date.	Result.	Inoculations of Guinea Pigs. Result.	Johnin Tests. Dates.	Vaccinations against Contagious Abortion.
Poppy ..	2	— But queried by M.H.	?	—		—										2.3.33—	Not done
Strawberry ..	5	— But queried by M.H.	—	—		—										2.3.33 ?	Vaccinated
Alice	5	— But queried by M.H.	—	—		—										2.3.33—	„
Cherry ..	5	+			+			—	—								„
Red Rose 29th	10	+			+			—	—							{3.2.30— 2.3.33—	Not done
Bessie ..	5	+			—								May, 1933	No tuberculosis	Negative		Vaccinated
Missie ..	3	—	+		+			—	—				Dec., 1933	„	„		Not done
Ruby ..	5	—	?	—		—										2.3.33—	Vaccinated
Primrose ..	10	—	?	Removed from the Herd, Johne's Disease Suspected												{3.2.30— 2.3.33+	Not done
Belle ..	4	—	?	—		+	—			—	—	—				2.3.33—	Vaccinated
Mina ..	2	—	?	+	—								May, 1933	„	„	2.3.33—	Not done
Bess	8	—	—	+	—								„ 1933	„	„	2.3.33—	„
Ruth II ..	5	—	—	+	—								„ 1933	„	„	2.3.33 ?	Vaccinated
Kathleen II ..	4	—	—	+	—								„ 1933	„	„	2.3.33—	Not done
Violet ..	6	—	—	+	+								„ 1933	„	„	2.3.33—	Vaccinated
Lena		—	—	+	+			—	—							2.3.33—	Not done
Dorothy ..	2	—	—	—		+	—			—	—	—				2.3.33—	„
Judy ..	5	—	—	—		+	—			—	—	—				2.3.33—	Vaccinated
Lily ..	4	—	—	—		+	—			—	—	—				2.3.33—	Not done

Abbreviations :—M.H. = Ministry of Health. — = Negative reaction to a tuberculin test. + = Positive reaction to a tuberculin test. ? = Doubtful reaction to a tuberculin test.

noted on the pleural surface of the diaphragm. One internal inguinal lymph gland showed one or two pin-head, whitish areas. These were not suggestive of tuberculosis. No other lesions were found.

Representative lymph glands were removed whole and placed in sterilised containers. Glands and tissues not intended for guinea-pig inoculation were examined at the slaughterhouse. The glands brought to the laboratory were dissected with sterile instruments and portions transferred into sterile dishes, ground up and suspended in normal saline solution. The remainder of the glands were placed in a cold store with a view to replacing guinea-pigs which might succumb to extraneous infection.

This technique was also adhered to in the ensuing examinations.

Two guinea-pigs were inoculated, one subcutaneously and one intraperitoneally, from each of the following groups of glands.

(1) Portal. (2) Mammary. (3) Bronchial. (4) Mesenteric. (5) Mediastinal. (6) Prescapular, popliteal, external iliac. (7) Internal inguinal. (8) Submaxillary, pharyngeal. (Sixteen guinea-pigs.)

With two exceptions, the guinea-pigs were killed and examined on July 31st, 1933, 79 days after inoculation. All were in good condition and none showed any lesions. The two guinea-pigs not examined had been inoculated inadvertently with material from a cow killed at a later date. These two guinea-pigs were killed at a later date and found free from tuberculosis.

Cow Mina.

Not pregnant.

Age, four years.

History. Negative in three tests, doubtful in January, 1933, and positive in March, 1933. Negative when retested in April.

Slaughtered and examined May 13th, 1933.

Post-mortem Examination.—The only abnormality encountered was the presence of a few strands of fibrin on the posterior lobe of the right lung. The cow was in good condition.

Guinea-pigs were inoculated in groups of two, one subcutaneously and one intraperitoneally, with suspensions of lymph glands grouped as follows:—

(1) Mesenteric. (2) Bronchial, mediastinal. (3) Prescapular, popliteal. (4) Mammary. (5) Iliac. (6) Submaxillary, pharyngeal. (7) Portal. (Fourteen guinea-pigs.)

The guinea-pigs inoculated subcutaneously with a suspension of submaxillary and pharyngeal glands and with mammary glands died on the day following inoculation and two more guinea-pigs were inoculated to replace them.

The guinea-pig inoculated subcutaneously with a suspension of mesenteric glands was found dead two days after inoculation. *B. welchii* infection was diagnosed. The guinea-pig inoculated intraperitoneally from the bronchial and mediastinal glands was found dead ten days after inoculation. *Post-mortem* examination revealed peritonitis, enlargement of the spleen, pleurisy and localised pneumonia. Smears from the peritoneal and pleural exudates showed numerous small Gram-negative bacilli. No lesion was found at the seat of the inoculation. The remaining twelve guinea-pigs were killed and

examined on July 31st, 1933, 78 days after inoculation. The *post-mortem* examinations upon ten of these revealed no lesions, but two guinea-pigs, inoculated from mediastinal and bronchial glands and from mesenteric glands respectively, showed enlargement of the spleen with hard, pearly-white, bead-like tubercles and small white areas were noted in the liver. No bacteria could be demonstrated in smears. Cultures from one of these guinea-pigs on plain agar yielded a growth of a bacillus which proved to be innocuous to a guinea-pig on subcutaneous inoculation. Cultures from the other remained sterile. A suspension of the spleen from each guinea pig was inoculated into two guinea-pigs, subcutaneously in the one and intraperitoneally in the other. These guinea-pigs, when killed and examined 68 days later, showed no evidence of tuberculosis, either by macroscopic, microscopic or cultural examination.

The four guinea-pigs in which lesions were encountered all occupied the same cage and were in all probability infected with pseudo-tuberculosis. This condition had been found in the stock from which these guinea-pigs were derived. In no instance was there enlargement or other other pathological change in the lymph glands at the seat of inoculation.

Cow Bessie.

Pregnant.

Age, five-and-a-half years.

History. Negative in five tests, positive in December, 1932, and negative in April, 1933.

Slaughtered and examined on May 29th, 1933.

Post-mortem Examination.—The only abnormal conditions found were a few fibrous deposits on the pleural surface of the diaphragm. The animal was in good condition.

Two guinea-pigs were inoculated, subcutaneously and intraperitoneally respectively, from each of the following groups of lymph glands :—

(1) Mesenteric. (2) Popliteal. (3) Prescapular and precrural. (4) Bronchial and mediastinal. (5) Submaxillary, pharyngeal. (6) Portal. (7) Mammary. (8) Internal iliac. (Sixteen guinea-pigs.)

One guinea-pig inoculated intraperitoneally with the submaxillary-pharyngeal group of glands was found dead three days after inoculation. Thirteen were killed and examined from September 5th, 1933, to September 7th, 1933, 98 days after inoculation, and were found normal and in good condition. The remaining two were spared to await weaning of litters. These were killed and examined on September 13th, 1933, and October 11th, 1933. Both were in good condition and showed no pathological changes.

Cow Violet.

Not pregnant.

Age, six years.

History. Negative in eight tests and positive in March, 1933, and April, 1933.

Slaughtered and examined on May 29th, 1933.

Post-mortem Examination.—The animal was in very good condition. The pericardium and diaphragmatic pleura showed lesions similar to those noted in the examination of the cow Bessie. Small discrete nodules were encountered in a precrural and a mesenteric lymph gland. These in no way resembled tuberculous lesions.

Guinea-pigs were inoculated, one subcutaneously and one intraperitoneally, from each of the following groups of glands :—

(1) Submaxillary, pharyngeal. (2) Mesenteric. (3) Mediastinal, bronchial. (4) Popliteal. (5) Mammary. (6) Prescapular, precrural. (7) Portal. (8) Two guinea-pigs were similarly inoculated with a suspension of a scraping from the pericardium. (Sixteen guinea-pigs.)

The guinea-pig inoculated subcutaneously from the mammary glands died seven days after inoculation.

On June 23rd, 1933, 25 days after inoculation, the guinea-pig inoculated intraperitoneally from the popliteal glands was found dead. *Post-mortem* examination revealed pleurisy, pericarditis, pneumonia, enlargement of the spleen and liver, subcutaneous œdema, and emaciation. Streptococci and acid-fast bacteria were seen in smears made from the pleura, but smears from other organs including the lymph glands at the seat of inoculation were negative. Two guinea-pigs and a rabbit were inoculated with suspensions of the material containing the acid-fast bacilli. The guinea-pigs showed no lesions when examined 79 days later. The rabbit was examined at the same date and was found to have suffered from a purulent pericarditis ; an abscess was also present at the seat of inoculation. No acid-fast bacteria were found in smears from the pus from either of the lesions.

Thirteen of the remaining 14 guinea-pigs were examined on September 5th, 6th and 7th, 68 to 70 days after inoculation. All were in good condition and showed no lesions. The remaining guinea-pig, killed and examined 92 days after inoculation, was also negative.

Cow Missie.

Not pregnant.

Age, four years.

History. Negative in four tests and positive in January, 1933, and April, 1933, tests. Found *in extremis* and slaughtered on December 18th, 1933.

Post-mortem Examination.—The pharyngeal region was œdematous, the ileum and jejunum were inflamed and their contents hæmorrhagic. The mesenteric lymph glands showed areas of congestion. The lesions noted were of an acute nature.

Guinea-pigs were inoculated, one subcutaneously and one intraperitoneally, from each of the following groups of glands :—

(1) Pharyngeal, submaxillary, parotid. (2) Prescapular, popliteal, precrural, internal inguinal. (3) Mediastinal, bronchial. (4) Mesenteric, inguinal, portal. (Eight guinea-pigs.)

One guinea-pig, inoculated from the mesenteric-inguinal-portal group of glands, was found dead on January 7th, 1934, 19 days after inoculation. Advanced pseudo-tuberculosis was diagnosed. No acid-fast bacteria could be demonstrated.

The remaining seven guinea-pigs were killed and examined on February 21st, 1934, 64 days after inoculation. All were in good condition and no pathological changes were encountered.

General Herd Tests, December, 1932, to October, 1933.

The test carried out in March was conducted by the Ministry of Health, and only the final results are recorded here. The tests upon animals which remained negative throughout are not recorded. (*See* Table 1 in text.)

12TH DECEMBER, 1932.

Name.	*Normal Skin Measurement in mm.*	*48 Hours.*		*24 Hours after Second Inoculation.*		*Result.*
		Description.	*Measurement.*	*Description.*	*Measurement.*	
Poppy	7	N.	10	N.	15	Interpretation queried by M.H.
Strawberry ...	5	N.	8	N.	13	„
Alice	7	N.	9	N.	16	„
Cherry	4·5	N.	15·5	D.	19·5	Positive
Red Rose 29th ...	6	D. and "feathery"	9	D. and "feathery"	14	„
Bessie ...	8	N.	18	N.	22	„
Missie	7	N.	11	N.	13	Negative
Ruby	6	N.	8	N.	11	„
Primrose ...	5	N.	7	N.	10	„
Belle	6·5	N.	8	N.	9·5	„
Mina	7	N.	10	N.	12·5	„
Bess	5	N.	7	N.	9·5	„
Ruth III ...	5	N.	8	N.	11	„
Kathleen II ...	7	N.	8	N.	10·5	„
Violet	6	N.	9	N.	9	„
Lena	7·5	N.	9·5	N.	11·5	„
Dorothy ...	7	N.	9·5	N.	10	„
Judy	6·5	N.	8	N.	9·5	„
Lilly	7	N.	9	N.	10	„

30TH JANUARY, 1933.

Poppy	7	N.	11	S.D.	16	Doubtful
Strawberry ...	5	S.D.	10	N.	13	Negative
Alice	7	N.	10	N.	13	„
Missie	7	N.	12	H.P.	21	Positive
Ruby	5	N.	11	T.	14	Doubtful
Primrose ...	4	S.D.	8	N.	13·5	„
Belle	7·5	N.	10	Soft	14	„
Mina	6	N.	9	D.	15	„
Bess	4	N.	9	N.	10	Negative
Ruth III ...	5	N.	9	N.	11	„
Kathleen II ...	6	N.	10	N.	14·5	„
Violet	5·5	N.	8	N.	12	„
Lena	6	N.	9	N.	11	„
Dorothy ...	6·5	N.	9	N.	11	„
Judy	6	N.	10	N.	12·5	„
Lilly	7	N.	12	N.	13	„

Name.	Normal Skin Measurement in mm.	48 Hours.		24 Hours after Second Inoculation.		Result.
		Description.	Measurement.	Description.	Measurement.	
6th March, 1933.						
Poppy	—	—	—	—	—	Negative
Strawberry ...	—	—	—	—	—	,,
Alice	—	—	—	—	—	,,
Ruby	—	—	—	—	—	,,
Belle	—	—	—	—	—	,,
Mina	—	—	—	—	—	Positive
Bess	—	—	—	—	—	,,
Ruth III ...	—	—	—	—	—	,,
Kathleen II ...	—	—	—	—	—	,,
Violet	—	—	—	—	—	,,
Lena	—	—	—	—	—	,,
Dorothy ...	—	—	—	—	—	Negative
Judy	—	—	—	—	—	,,
Lilly	—	—	—	—	—	,,
10th October, 1933.						
Poppy	8	N.	11	N.	15	Negative
Strawberry ...	6	N.	9	N.	11	,,
Alice	7	N.	9	N.	14	,,
Ruby	7	N.	10	N.	11	,,
Belle	6	D.T.	18	N.	21	Positive
Dorothy ...	8	S.D.	14	N.	23	,,
Judy	8	D.	15	Hard	20	,,
Lilly	9	N.	15	Hard	18	,,

Abbreviations :—D. = Diffuse.
S.D. = Slightly diffuse.
H. = Heat.
P. = Pain.
T. = Tenderness.
M.H. = Ministry of Health.
N. = Negative reaction.

Johnin Test. February 3rd, 1930.

The following were subjected to the johnin test, 0·2 c.c. of johnin being inoculated intradermally in the caudal fold.

Red Rose 29th ...	... Negative.
Primrose	... ,,
Bess	... ,,
Violet	... ,,

JOHNIN TEST. MARCH 2ND, 1933.

Name.	*Description of Swelling after 48 Hours.*	*Description of Swelling after 72 Hours.*	*Result.*
Mina	Appreciable	Appreciable	Negative
Belle	Pea	Pea	„
Primrose	Twice. Oedema	Twice. Oedema	Positive
Poppy	Nodule	Nodule	Negative
Ruby	„	„	„
Alice	Nut	Nut	„
Strawberry	Feathery swelling	Feathery swelling	Doubtful
Lilly	Just appreciable	Just appreciable	Negative
Judy	Pea	Pea	„
Lena	Nodule	Nodule	„
Violet	Just appreciable	Just appreciable	„
Dorothy	? appreciable	? appreciable	„
Kathleen	Slight thickening	Slight thickening	„
Ruth	Diffuse thickening	Diffuse thickening	Doubtful
Bess	Pea	Pea	Negative

TUBERCULIN TEST. APRIL 20TH, 1933.

Intradermal tuberculin test upon animals removed from herd—0·1 c.c. intradermal tuberculin (Cambridge).

Name.	*Normal Skin in mm.*	48 *Hours.*		24 *Hours after Second Inoculation.*		*Result.*
		Description.	*Measurement.*	*Description.*	*Measurement.*	
Kathleen ...	8	Bean	9	Bean	13	Negative
Lena	8	Painful	15		25	Positive
Bess	4	Bean	8	Bean	12	Negative
Bessie	8	Bean	12	Bean	15	„
Mina	8	Bean	9	Bean	12	„
Red Rose 29th ...	6	Painful	11		20	Positive
Violet	7	S.D.	14		22	„
Missie	8	Bean	11	D. & P.	19	„
Cherry	6	Not reinoculated	30		29	„
Ruth III	7	Bean	11	Bean	13	Negative

Abbreviations :—S.D. = Slightly diffuse. D. & P. = Diffuse and painful.

TUBERCULIN TEST. OCTOBER 26TH, 1933.

Four animals removed from the herd after re-acting to the intradermal test were subjected to the subcutaneous test on 26.10.1933. Temperatures were taken twice on 24.10.1933 and at intervals of 3 hours on 25.10.1933. 4 c.c. of dilute tuberculin (Cambridge) was inoculated at 10 p.m. on 25.10.1933, and temperatures were again taken at 3 hour intervals on 26.10.1933.

0·1 c.c. of intradermal tuberculin (Cambridge) was inoculated at 4 p.m. on 26.10.1933.

	Missie.		*Cherry.*		*Lena.*		*Red Rose 29th.*	
	Pre-Inoculation.	*Post-Inoculation.*	*Pre-Inoculation.*	*Post-Inoculation.*	*Pre-Inoculation.*	*Post-Inoculation.*	*Pre-Inoculation.*	*Post-Inoculation.*
10 a.m., 24.10.33	103·6		101·4		102·2		101·4	
4 p.m., 24.10.33	102·9		101·2		101		101·1	
7 a.m.	102·6	102	101·1	101·3	101	102	100·9	101·4
10 a.m.	102·8	101·8	102·3	101·5	101·7	101·9	101·6	100·3
1 p.m.	103	101·9	101·4	101·4	101·6	102·6	101·4	101·4
4 p.m.	103·6	102	102	102·8	102·0	103	102	102·7
7 p.m.	102	Calving	101	101	101·2	101·6	101·2	102·3
10 p.m.	102·2	103·6	101·6	101·2	102·4	102	102·6	101·7

TUBERCULIN TEST. OCTOBER 26TH, 1933.

Intradermal test carried out at the conclusion of subcutaneous test. 0·1 c.c. intradermal tuberculin (Cambridge) was inoculated in the neck.

Name.	*Normal Skin Fold in m.m.*	*48 Hours.*		*24 Hours after Second Inoculation.*		*Result.*
		Description.	*Measurement.*	*Description.*	*Measurement.*	
Missie... ...	6	Pea	10	Pea	11·5	Negative
Cherry ...	7	Bean	13	Bean	14	„
Lena	7	Bean	10	Bean	15	„
Red Rose 29th	8	Diffuse	11	Bean	14	„

TUBERCULIN TESTS. NOVEMBER 17TH, 1933.

Intradermal tests upon four animals which had given a positive reaction in the general herd test in October, 1933. Two brands of tuberculin were used, the inoculations being made simultaneously on the same side of the neck and about 10-in. apart.

(1) *Broth tuberculin (Cambridge).*

Name.	*Normal Skin Fold in mm.*	*48 Hours.*		*24 Hours after Second Inoculation.*		*Result.*
		Description.	*Measurement.*	*Description.*	*Measurement.*	
Dorothy ...	8	Bean	12	Bean	14	Negative
Belle ...	7	Bean	13	V.S.D.	16	„
Judy	8	Small bean V.S.D.	11	Bean	15	„
Lilly	9	Bean	14	Bean	16	„

(2) *Synthetic Medium Tuberculin.*

Dorothy ...	7	Inapp.	7	Barely appr.	7½	Negative
Belle ...	7	Pea	8	Small bean	10	„
Judy	7	Pea	8	Bean	12	„
Lilly	7	Barely appr.	8	Barely appr.	9	„

Abbreviations :—V.S.D. = Very slightly diffuse. Appr. = Appreciable. Inapp. = Inappreciable.

TUBERCULIN TEST. DECEMBER 18TH, 1933.

Four animals which reacted to the intradermal test (General herd test) in October, 1933, were subjected to the subcutaneous test, 4 c.c. of tuberculin (Cambridge) being injected.

Temperatures were taken at 3-hour intervals on 18.12.33, the injection was made at 10 p.m. on that day, and temperatures were again taken at corresponding intervals on the following day.

	Judy.		*Belle.*		*Dorothy.*		*Lilly.*	
	Pre-Inoculation.	*Post-Inoculation.*	*Pre-Inoculation.*	*Post-Inoculation.*	*Pre-Inoculation.*	*Post-Inoculation.*	*Pre-Inoculation.*	*Post-Inoculation.*
7 a.m.	101·6	100·9	101·4	101	102	101·9	101·6	101·5
10 a.m.	102	102·1	102	101·9	102	102·4	102	102·8
1 p.m.	100	100·8	100·4	101·6	101·6	102·1	101·1	102·8
4 p.m.	101	102·0	101	101·5	102·1	102·6	102·3	103
7 p.m.	100·9	100·8	100·9	100·9	102·1	102·5	100·4	100·6
10 p.m.	101·3	101·5	101	101·0	101·1	102·8	100·4	101·8
		Neg.		*Neg.*		*Neg.*		*Neg.*

Vaccinations against Contagious Abortion.

The vaccine consisted of a saline suspension of *Br. abortus* equivalent in opacity to a suspension of *B. coli* containing 757 million bacteria per c.c. The suspension was prepared from 48-hour cultures on liver agar and in every case the dose of vaccine consisted of 2 c.c. inoculated subcutaneously.

Name of Heifer or Cow.	*Dates when Vaccinated.*	*Name of Heifer or Cow.*	*Dates when Vaccinated.*
Poppy	Never done	Primrose	Never done
Strawberry	25. 9.31	Belle	26. 8.31
	2.11.31		2.11.31
	6. 1.32		6. 1.32
	23. 3.32		23. 3.32
	28. 5.32		28. 5.32
Alice	25. 9.31	Mina	Never done
	2.11.31	Bess	Never done
	6. 1.32	Ruth III	26. 9.31
	23. 3.32		2.11.31
	28. 5.32		6. 1.32
Cherry	10. 7.31		23. 3.32
	26. 8.31		28. 5.32
	31.10.31	Kathleen II	Never done
	2.11.31	Violet	26. 9.31
	6. 1.32		2.11.31
	23. 3.32		6. 1.32
	28. 5.32		23. 3.32
Red Rose 29th ...	Never done		28. 5.32
Bessie	25. 9.31	Lena	Never done
	31.10.31	Dorothy	Never done
	2.11.31	Judy	2.11.31
	6. 1.31		6. 1.32
	23. 3.32		23. 3.32
	28. 5.32		28. 5.32
Missie	Never done	Lilly	Never done
Ruby	2.11.31		
	6. 1.32		
	23. 3.32		
	28. 5.32		

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STUDIES ON BOVINE MASTITIS.

X.—The Value of Field and Laboratory Tests for the Diagnosis of Chronic Streptococcus Mastitis.

By S. J. Edwards.

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This paper may be regarded as a continuation of that published by Minett, Stableforth and Edwards (1930) on the diagnosis of chronic streptococcus mastitis. The object of the present investigations was to obtain further experience of the indirect methods dealt with in the 1930 paper, but using as a basis of comparison the improved selective medium of which an account was given in the last paper of this series (S. J. Edwards, 1933). This further experience was required because of the desirability of obtaining more exact information as to the extent to which relatively simple tests can be used by veterinary practitioners or owners for diagnosing cases of latent infection, and to what extent laboratory aid has to be relied upon for this purpose.

Experiments intended to select the most appropriate methods for diagnosing of latent infections on a large scale have also been described during 1933 by Rosell, Rosell and Miller, Hadley and Frost, and by Hucker, and in 1932 by Seelemann. With the exception of Seelemann, these authors attach great importance to the value of indirect methods of diagnosis. The catalase and chloride tests, for instance, are regarded as very efficient, since they are able to reveal as many as 80 per cent. of cases. Rosell considers that 85 to 90 per cent. of infected cows can be detected by the brom-thymol-blue test when the reagent is added to milk freshly taken from the individual quarters. Hucker finds the chloride test somewhat difficult to interpret, since the end point may be vague and there is some doubt as to what should be taken as normal values. He also finds the catalase test too delicate in the sense that some cows with healthy udders may be condemned.

In connection with most of the investigations mentioned, the cultural examination has been made on the surface of media and, except in the case of Seelemann, the results were found to be inferior to those of the indirect tests used. It should be noted, however, that Hucker used as seed material one loopful of milk, which is, of course, less satisfactory than the centrifuged deposit of, say, 10 c.c. of milk.

At present the only means available for combating chronic streptococcus mastitis is to prevent its dissemination from infected to healthy cattle. That the disease *can* be controlled and finally eliminated in this way has been shown conclusively by Minett, Stableforth and Edwards (1933).

The Herds Examined.

In this investigation milk samples from 809 cows belonging to 18 herds have been examined on one occasion by the tests mentioned in the next section. In general, the history given by the owners was that there was no udder disease of an acute nature, and that the only disturbances noted were periodic swelling of the quarters of certain cows and changes in the milk leading to the establishment of a " light " quarter. This is, of course, the usual history that would lead one to suspect the presence of chronic streptococcus mastitis in the herd.

The results showed that 295 cows, or 36·4 per cent., were infected in at least one quarter, a figure which agrees closely with results obtained previously in this Institute. The incidence of infection in the different herds varied between 10 and 84 per cent. Milking was carried out by hand in 13 herds and in the other five by machine, but there is no evidence that the incidence of mastitis was greater in the machine-milked herds.

Out of 619 strains of mastitis Group I streptococci (*i.e., Str. agalactiæ*) isolated from the milk samples, 144 were non-hæmolytic and the high proportion of 475 were beta-hæmolytic in ox-blood agar. Other mastitis bacteria, *e.g., C. pyogenes, Bact. coli* and certain staphylococci were not encountered. Mastitis streptococci belonging to Group II were grown from the udders of 13 cows in seven herds. Six of these cows had suffered from clinical mastitis due to these organisms, while in the remainder there were only slight changes in the milk.

The fact that Group I streptococci were nearly the only pathogenic organisms met with shows once again that chronic streptococcus mastitis is the most prevalent form of udder disease.

Methods of Diagnosis Used.

Before the milk was taken the udder was washed in the ordinary way and wiped with a cloth. The milk of each quarter was examined and the methods used may be conveniently described under two headings: (i) those used at the farm, and (ii) those carried out in the laboratory on milk samples stored overnight at a low temperature. For this purpose, 15 c.c. of fore-milk from each quarter were drawn into a sterile tube, and then, in order to test the value of the method, a mixed sample containing equal amounts of milk from each of the quarters was taken into another tube.

(i) (*a*) *History of the Cow.*—The owner was questioned as to previous history of disease. Any account of abnormalities such as clots in the milk, swelling of the udder, and reduction in the milk yield, not due to other specific causes, were taken as indicating infection.

(*b*) *Inspection of the Milk.*—Gross changes in the milk were looked for, such as the presence of yellowish clots or a pronounced watery appearance. Fore-milk from each quarter was drawn

into a shallow dish with blackened surface, sufficient to give a thin layer. Four such dishes were attached to a plate so that all quarters could be examined simultaneously. Quite frequently it was found that the milk from infected quarters only contained one or two small clots.

(*c*) *Estimation of the Reaction of the Milk.*—Papers impregnated with a saturated solution of brom-cresol-purple were used, since this is a simple way of estimating the reaction. The papers, in a suitable holder, were dipped into the milk of each quarter in the dish, and the reaction was read after they had drained for a minute or two. With a little practice, the purplish tint given by an abnormally alkaline milk could be readily distinguished from the yellowish-green colour of normal milk. As a guide to the normal colour, the milk of a first-calf heifer may be tested.

(ii) (*a*) *Estimation of the Amount of Deposit.*—For this purpose, 10 c.c. of milk were centrifuged at a speed of 2,500 revs. per minute for five minutes in a modified Trommsdorff tube, as described by Minett, Stableforth and Edwards (1930). Deposits of 0·01 c.c. or over were regarded as abnormal. Actually, in a small proportion of cases a deposit may be classed as abnormal, although its amount is less than 0·01 c.c., owing to the alteration in its colour and its sharp separation from the supernatant. Nevertheless the arbitrary standard of 0·01 c.c. has been used, because this gives a more definite standard of comparison than qualitative changes, and more particularly since the sediment from quarters free from pathogenic bacteria is almost invariably less than 0·01 c.c.

(*b*) *Cultural Examination.*—This was the final basis of comparison in assessing the value of other tests, the selective medium previously referred to being used. Centrifuged deposits were mixed with about 2 c.c. saline, one half of the mixture being placed in one plate and the other half diluted with 9 c.c. of saline of which 1 c.c. was added to a second plate. After 24 to 48 hours at 37° C. beta-hæmolytic colonies can be easily recognised. Non-hæmolytic colonies resembling those of streptococci were picked into serum broth and their group determined by transfer to methylene-blue milk, mannite and salicin.

In a number of cases the values of deep and of surface cultivation were compared, about one-half of the milk deposit being spread on the surface and the other half being used for deep plating. With surface cultivation the medium was similar except that it contained 2·5 per cent. ox-blood agar instead of 5 per cent. and the agar was poured to a depth of 1 mm. (*i.e.*, about half the depth used in the deep method) and incubated overnight to dry.

Results.

A. Milk Samples from Individual Quarters.

In considering the results of the indirect tests and their relationship to cultural examination, milk samples from individual quarters have been arranged in two series.

Firstly, samples from cows in full milk are considered separately from those of drying cows, because the physiological

changes which take place in the drying udder by themselves alter the composition of the milk.

Secondly, the samples have been placed in two groups: (*a*) those free from Group I mastitis streptococci, and (*b*) those containing these organisms.

TABLE I.

THE EXAMINATION OF MILK SAMPLES FROM INDIVIDUAL QUARTERS AND OF MIXED SAMPLES.

	Stage of Lactation.	*Cultural Examination.*	*No. of Samples.*	*History of Diseases.* +	—	*Visible Changes in Milk (Dish Test).* +	—	*Reaction.* +	—	*Deposit.* +	—
Quarter Samples	Full Milk	—	2,284	2	2,282	4	2,280	137 (6)	2,147 (94)	123 (5·4)	2,161 (94·6)
		+	528	97 (18·3)	431	146 (28)	382	255 (48·3)	273	262 (49·6)	266
	Drying	—	132	1	131	11 (8·3)	121	112 (85)	20	39 (30)	93
		+	91	46 (50)	45	46 (50)	45	85 (93)	6	76 (83)	15
Mixed Samples	Full Milk	—	429	2	427	5	424	———		18 (4·2)	411 (95·8)
		+	238	54 (22)	184	92 (38)	146	———		90 (38)	148
	Drying	—	25	1	24	4 (16)	21	———		12 (48)	13
		+	24	10 (41·7)	14	15 (62·5)	9	———		19 (79·1)	5

(Figures in brackets denote percentages.)

Cows in Full Milk.—As shown in Table I, 2,284 healthy quarters were examined with the following results. In only two cases was there a history of disease, and in only four cases was the milk visibly altered. The reaction to brom-cresol-purple was normal with 2,147 samples (94 per cent.) and excessively alkaline with 137 (6·0 per cent.). With samples from 2,161 quarters (94·6 per cent.) the amount of deposit was within normal limits and in 123 (5·4 per cent.) 0·01 c.c. or over.

With the 528 infected quarters symptoms of disease had been seen by the owner in 97 cases (18·3 per cent.) and physical changes in the milk were present in 146 instances (28 per cent.). The reaction was normal in 273 (51·7 per cent.) and slightly or excessively alkaline in 255 (48·3 per cent.). With 266 samples

(50·4 per cent.) there was a normal deposit, while 262 samples (49·6 per cent.) gave abnormal amounts.

A comparison which is not without value may be made between the results of the reaction and deposit tests in the present series and in the series reported by Minett, Stableforth and Edwards (1930), since the basis of comparison in the series was not dissimilar. In the case of the latter, there were 485 milk samples from healthy quarters where the reaction to brom-cresol-purple papers was normal in 449 (92·5 per cent.) and the abnormal in 36 (7·5 per cent.). Of 326 samples from streptococcus-infected quarters, the reaction was definitely alkaline in 76 (23 per cent.), judged to be intermediate or slightly alkaline in 133 (40·7 per cent.) and normal in 117 (35·8 per cent.). As to the deposits, of 485 culturally negative samples, the amount was less than 0·01 c.c. in 463 (95·4 per cent.) and more than this in 22 (4·5 per cent.). With 326 samples from infected quarters, the deposit was 0·01 c.c. or more in 199 (61 per cent.) and less than this amount in 127 (39 per cent.).

Thus with healthy quarters the results are very nearly the same in both series of observations. On the other hand, with the infected quarters in the present series a smaller proportion were abnormal to the reaction and deposit tests.

Cows in the Drying Stage.—Chronic streptococcus mastitis frequently does not make its appearance clinically until near the end of the lactation period, hence abnormalities in the milk would be expected in a larger proportion of infected cows at this stage than with infected cows in full milk. Thus of 91 infected quarters, 46 (50 per cent.) showed visible changes in the milk, but at the same time the milk of eleven (8·3 per cent.) healthy quarters showed similar changes. History of disease was notified by the owner in 50 per cent. of the infected quarters. An abnormal reaction to brom-cresol-purple was given by over 90 per cent. of samples from infected cows, but the proportion was nearly as high with the samples from healthy cows. The deposit test gave better results. Thus, of samples from healthy quarters 39 (30 per cent.) showed deposits of 0·01 c.c. or over, whereas with 83 per cent. of infected samples the deposits were excessive.

B. Mixed Milk Samples from Individual Cows.

In mastitis the whole udder is not usually involved, so that milk from infected quarters is likely to be diluted with normal milk. Reactions of mixed samples, therefore, were not taken, but as centrifuged sediments were to be cultured the opportunity was taken to note the amounts.

Cows in Full Milk.—Of 429 samples from healthy cows, 411 (95·8 per cent.) gave deposits less than 0·01 c.c. and 18 (4·2 per cent.) 0·01 c.c. or over. Of 238 samples from cows infected in at least one quarter, 90 (38 per cent.) gave abnormal deposits and 148 (62 per cent.) normal amounts.

Cows in Drying Stage.—Of 25 samples from healthy cows, twelve (48 per cent.) gave excessive deposits, while of 24 samples

from infected cows 19 (79 per cent.) gave abnormally high amounts.

Table II.

Cultural Examination of Samples of Mixed Udder Milk and of Corresponding Samples from Individual Quarters.

Number of Infected Cows.	*Number of Quarters Infected in each Cow.*	*Mixed Sample.* +	—
45	4	45	0
35	3	34	1
60	2	58	2
79	1	62	17
Total 219		199 (91)	20 (9)

(Figures in brackets denote percentages.)

Value of Mixed Samples for Cultural Diagnosis.

Table II shows the relation between the cultural findings with mixed milk samples and with the corresponding quarter samples from individual cows. Thus, of 219 infected cows, cultural examination of mixed samples revealed mastitis streptococci in 199 (91 per cent.). Of the 20 cows from the milk of which these organisms were not grown, 17 were infected in a single quarter. Of these 17, non-hæmolytic streptococci were present in the milk of ten and small numbers of beta-hæmolytic streptococci were present in the remainder. This suggests, therefore, that cultural examination of a mixed sample can reveal nine out of ten cows with chronic streptococcus mastitis and that failures are only to be expected when the disease is confined to one quarter and due either to non-hæmolytic forms or to beta-hæmolytic forms, present at the time in small numbers.

The Value for Diagnosis of Individual and of Combined Tests.

Cows in Full Milk.—The results in Table I show that individually the indirect tests are of somewhat limited value for diagnosis. Thus, as previously mentioned, visible change in the milk was observed in only 28 per cent. of the infected cows, and in only 18·3 per cent. did the owner give any history pointing to disease. By the reaction and deposit tests singly, not more than about 50 per cent. of the infected quarters were abnormal, while of the healthy quarters 6·0 per cent. were giving excessively alkaline milk and 5·4 per cent. showed high deposits. It follows from this that abnormality to one or other of these two tests would not *necessarily* indicate infection.

Table III.

Value of Indirect Tests in Combination.

		2,284 *Healthy Quarters.*		528 *Infected Quarters.*	
All test negatives		2,068 (90·6)		202 (38)	
One or more tests positive		216 (9·4)		326 (62)	
Dish Test	+	4	47 (2)	146	219 (41)
Reaction and Deposit	+	43		73	

(Figures in brackets denote percentages.)

Table III shows the results obtained when a combination of indirect tests is considered. Thus, of 2,284 samples from healthy quarters of cows in full milk, 2,068 (90·6 per cent.) were normal to all tests and 216 (9·4 per cent.) showed abnormality to one or other of the tests used. With samples from 528 infected quarters, 202 (38 per cent.) were normal and 326 (62 per cent.) showed abnormality of one sort or another. If, therefore, by indirect tests, one were to examine milk samples taken at random from cows said to be in full milk, and if reliance were placed solely on abnormality to one or other of them as indications of infection, only 326 (62 per cent.) of quarters containing the streptococci of chronic mastitis would be detected, while 216 (9·4 per cent.) of quarters free from these organisms would be similarly classed. This over-estimate of the incidence of chronic streptococcus mastitis is mainly due to results given by the reaction or deposit tests. In order to avoid this over-estimate attention may be paid in the first place to the dish test. If there are visible changes in the milk by this test, the existence of chronic streptococcus mastitis is practically always indicated. Similarly, if both reaction and deposit tests are positive the result almost certainly indicates infection. Taken individually, however, these tests cannot be regarded as conclusive evidence of disease, since, as shown above, one or the other of these tests may be positive in 5 or 6 per cent. of culturally negative milks. When the figure, 216, in Table III is analysed in the light of the foregoing, it will be seen that the over-estimate is reduced to 47; that is to say, in only 2 per cent. of 2,284 samples from healthy quarters would an incorrect diagnosis have been made. When this reasoning is applied to the 528 infected quarters. it will be seen that, whereas one or more tests were positive in 62 per cent. of cases, it was only in 41 per cent. of the total that evidence pointing to infection was definite.

Drying Cows.—Here, indirect tests give no information of value as to the presence or absence of infection. Some reliance may be attached to the presence of clots in the milk, but a watery condition of the sample should not suggest disease. Hence, the only proof of the existence of infection in cows stated to be nearing

the end of lactation can be derived from a cultural examination of the milk.

Comparative Value of Surface and Deep Plating.

In the first place comparison was made with milk samples from 370 infected quarters. In all of these streptococcus colonies were recognised by surface cultivation, except in 64 (17 per cent.). Of these 64 samples, 14 showed 50 to 200 (average, 100) beta-hæmolytic colonies in the corresponding deep plate and 32 less than this number, while 18 contained non-hæmolytic streptococci.

In general, the recognition of large numbers of surface colonies of beta-hæmolytic streptococci is not difficult owing to the uniform appearance of the colonies and the slight clearing around, but it is often impossible to recognise small numbers, particularly when other dye-resistant organisms predominate. Although surface cultivation on blood agar may be said to be a more expeditious way of examining milk samples—and even this is questionable when reasonably good facilities exist—without considerable experience it cannot afford such definite assurance of the presence of streptococci as deep cultivation.

The Diagnosis of Latent Streptococcus Infections in Practice.

When a diagnosis of chronic streptococcus mastitis is to be made in a herd the procedure followed will depend to some extent on the local conditions and facilities available for carrying out field tests. The owner may desire that these tests should be employed to the full as a preliminary measure, or, on the contrary, he may be anxious for a final diagnosis as quickly as possible, and this can only be got by laboratory aid. When the second plan is to be adopted there should first of all be made a list of all the cows in milk, and with each cow a note made of the number of calvings, the date of the last calving, and of any history of udder disease which the owner or herdsman may furnish. After wiping the udder with a damp cloth, a little of the fore-milk from each quarter may be examined in a black dish for clots and, at the same time, the reaction may be taken with brom-cresol-purple paper. Cows which show clots by the dish test may be regarded as infected, and this is nearly always confirmed by the increased alkalinity of the milk. From all cows other than those which show clots, 15 c.c. of fore-milk should be taken from individual quarters into sterile tubes. The samples are immediately set aside in a cool place and despatched as soon as possible by rail or post to the laboratory for further examination. In the laboratory, the samples are shaken and 10 c.c. amounts poured into the special tubes for centrifuging at 2,500 revs. per minute for five minutes. With cows in full milk, a deposit of 0·01 c.c. or over indicates infection without further examination if they have previously shown an alkaline reaction. The remaining deposits should be subjected

to cultural examination in the selective medium. At the first test, it is possible to reduce the work by making a cultural examination of mixed samples or of quarter samples on the surface of the medium, but, as shown earlier in this paper, the results are not so conclusive as when deep plating of quarter samples is practised.

The results of the laboratory examination will provide the final information required for the division of the herd into healthy and infected sections, and cows found to be infected should thenceforward be milked last. After this, heifers calving for the first time need not be examined, but the milk of all cows calving subsequent to the initial test should be examined, though not sooner than seven days after calving; similarly, new purchases should be tested before admission to the main milking herd. A really good cultural test can be relied upon to give a final answer in the great majority of cases, but it is advisable to repeat the examination of the healthy group after three months. A few further tests can also be carried out at intervals of three to four months on samples of mixed quarter milk. As an added precautionary measure in the control of the disease, it is always a good plan to milk cows in the order of age, beginning with the younger ones.

If there are reasons which make an extended use of field test desirable, as for instance when access to a laboratory is difficult or when the herd is large, quarter samples should be examined in the cowshed as in the above method. By way of confirmation there is no reason why the sediment test in the field, under conditions as comparable as possible with those indicated above, should not be used. A repetition of these tests on several occasions will help to confirm doubtful cases and to detect fresh cases of infection. As in the method first described, quarter samples of the remaining cows in milk should be sent to the laboratory. Finally, there is no reason for thinking that control work need be a costly undertaking, and ordinarily equipped laboratories should be able to undertake their part of the work at a comparatively low cost.

Discussion.

When the value of indirect methods of diagnosis is to be decided, the first essential is that comparison should be made with a standard test of the utmost reliability for revealing pathogenic bacteria. Naturally, this is the only true index of infection, and it is obvious that unless the standard cultural test *is* thoroughly reliable the efficiency of indirect methods will appear to be exaggerated, since only the more advanced cases will be detected. Again, it is important that the whole deposit from 10 c.c. of milk be cultured. These simple and important facts have, of course, been recognised by a number of workers, and one of the main objects of the work of this Institute has been to devise a selective

medium capable of detecting very small numbers of mastitis streptococci.

In the present investigation, the choice of indirect methods has been intentionally confined to those which could be easily used by a veterinary practitioner or an owner, and there seemed to be no advantage in including other laboratory tests, *e.g.*, estimations of catalase or chlorides. However, in addition to the reaction, sediment and cultural tests, note has been taken of the history of the cow, and milk from each quarter has been examined for clots in a black dish. The udder was not palpated as a routine, since almost all observers agree that this operation can only reveal a proportion of cows in advanced stages. Since, too, it is agreed that a microscopical examination of milk sediment for streptococci is of no great value, this step also was omitted.

In a general way, whatever their advantages may be, indirect methods suffer from two serious defects. Firstly, they are incapable of detecting more than a moderate percentage of infected animals, the failures being in part accounted for by those in the early stages of infection. Secondly, as has been shown in this paper, indirect methods give misleading results in a not insignificant proportion of animals. This can be explained either because healthy animals may be approaching the end of lactation, although still regarded by the owner as in full milk, or because they may be suffering from forms of udder disease due to staphylococci or streptococci other than *Str. agalactiæ*. As there is no reason for regarding such forms as infectious and as many cases are mild and clear up on their own account, it would obviously be unsound to place them with animals infected with *Str. agalactiæ* and thus give them the opportunity of contracting this more serious form. It should not be implied, however, that certain indirect tests in suitable combination are incapable of giving reliable indication of chronic streptococcus mastitis. Thus, it has been shown that the presence of clots in the fore-milk, when accompanied by an alkaline reaction with brom-cresol-purple paper, may be regarded as definite evidence of this disease. An abnormal reaction alone is less satisfactory and is only to be taken as evidence of disease when, after centrifuging 10 c.c. of milk under standard conditions, the deposit measures 0·01 c.c. or over. This additional test of course requires the provision of a suitably designed centrifuge, but it might be carried out in large herds by means of a Gerber machine adapted for the purpose. A single application of three tests, *viz.*, examination of the milk in a black plate, as well as by the reaction and deposit tests, gives a reliable indication with 41 per cent. of the cows infected with chronic streptococcus mastitis. By repeating the tests two or three times, it is probable that a somewhat larger number of positive results would be obtained.

Summary.

1. To obtain further experience of the relative values of field and laboratory tests for the diagnosis of chronic streptococcus mastitis, 809 cows in 18 herds were examined. Of these, 295 (36·4 per cent.) were infected in at least one quarter with mastitis Group I streptococci (*Str. agalactiæ*).

The field tests included the history of the cow, a test of milk of individual quarters for its reaction and the presence of clots. In the laboratory, the amount of deposit obtained by centrifuging was noted and a cultural examination made in or on a highly selective blood agar medium, this being the basis of comparison. The value of cultural examination of mixed samples from the four quarters was also tested.

2. Of 528 infected quarters of cows said to be *in full milk*, 62 per cent. showed abnormalities to one or other of the indirect tests, but by the same criteria 9·4 per cent. of milk samples from 2,284 healthy quarters also showed abnormalities.

To avoid misleading results with quarters free from *Str. agalactiæ*, it is preferable to accept as evidence of this infection only instances where both reaction and deposit tests are positive. When clots are present in the fore-milk the cow may be regarded as infected, and this, when taken in conjunction with positive results to both reaction and deposit tests, is capable of revealing 41 per cent. of cows infected with latent mastitis. In cows in the *drying stage*, cultural examination alone is of value.

3. With 219 infected cows examination of the mixed milk samples by deep plating showed the presence of streptococci in 91 per cent. The method was liable to fail only when non-hæmolytic forms were present, usually in one quarter, or when the cow was infected with small numbers of beta-hæmolytic streptococci. In 83 per cent. of infected quarters streptococci could be detected by surface cultivation.

4. Procedures are outlined for the diagnosis of the disease in practice. The first plan aims at a quick and final diagnosis by cultural examination, while the second embraces a more extended application of field tests. It should be emphasised, however, that simple field tests can only serve for a preliminary survey of the herd, and the full extent of the disease can only be determined by a cultural examination of milk from individual cows.

My thanks are due to Dr. F. C. Minett for his advice and helpful criticism.

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BACTERIUM PFAFFI (HADLEY) INFECTION IN CANARY BIRDS.

By Norman Hole.

From the Laboratory of the Ministry of Agriculture and Fisheries, Weybridge.

In 1931, an epizoötic occurred in a small aviary in Kent, in which Canaries, Goldfinch Mules, Greenfinches, Linnets, Crossbills, Pekin Robins, Red Crested Cardinals, Waxbills, Siskins and other finches were kept; seven out of eight canaries died within a month, and, in the owner's words, the great majority of the other types died too.

All the birds were kept either singly or two in a cage. The only symptom noted was " moping "; no diarrhœa occurred.

Laboratory Investigations.

Four dead canaries were received. *Post-mortem* examinations showed hyperæmia of the serous membranes, inflammation of the duodenal mucosa, and enlarged and very congested spleens. The outstanding features, however, were pin-head yellowish-white foci in the liver. They were present in only three of the birds, and the appearance resembled miliary tuberculosis. No tubercle bacilli could be detected in smears of the necrotic material stained Ziehl-Neelsen. Cultures were obtained from the liver, spleen and blood on agar-agar and in peptone broth after incubating ærobically for 24 hours at 37° C. These cultures appeared to be quite pure, but, for identification work, single colonies were picked up from plates and subcultured.

Morphology.—The organism was rather pleomorphic. The most common form was that of a small cocco-bacillus, but larger bacillary forms were seen, and rarely these latter were joined in a chain of 3-5 elements. These variations were repeatedly found in cultures of undoubted purity. The organism was Gram-negative and non-motile.

Culture.—Twenty-four hour agar slopes of the organism were similar to, but slightly heavier than, *B. pullorum*. Older cultures showed a tendency to become waxy. In gelatin stab or shake the heaviest growth occurred near the surface. There was no liquefaction. On potato growth was very poor and almost invisible even after several days. Broth cultures were sometimes uniformly cloudy and sometimes contained a deposit. Later work showed this to be due to a rough-smooth variation. When subcultured in broth pure rough cultures gave a uniform turbidity; pure smooth cultures gave a deposit and clear supernatant fluid.

Biochemical Reactions.

Sugars.—(Andrade Indicator.)

No gas formed in any medium.

Acid readily formed in arabinose, dextrin, galactose, glucose, lævulose, maltose, mannite, rhamnose and xylose.

Acid slowly formed (seven to eight days at 37° C.) in adonite and salicin. Growth occurred, with no acid formation, in dulcite, inosite, inulin, lactose, raffinose, saccharose and sorbite.

Other Tests.—H_2S was not produced in lead acetate broth.

No indol was formed.

Litmus milk at first remained unchanged but became alkaline after five days.

Serology.—No agglutination occurred with *Bact. pullorum*, *Bact. enteritidis* and *Bact. ærtrycke* positive sera.

Pathogenicity.

All inoculations were made with 24 hour broth cultures. The organism was recovered from the tissues in every case, unless otherwise stated, if death occurred.

Fowls resisted large doses either by the subcutaneous or intravenous routes.

Pigeons were not tested until the organism had been subcultured for two years. They then resisted.

Rabbits and *Guinea-pigs* succumbed in four to five days to 0·1 c.c. intravenous or intraperitoneal injection, but resisted 0·5 c.c. given subcutaneously. The only lesion found in these animals was enlargement and congestion of the spleen.

Canaries.—Minute doses (0·01 c.c.) inoculated subcutaneously caused death in every case in two to three days. Doses of 0·1 c.c. by the mouth caused death in seven to eleven days. No canary survived subcutaneous or direct oral administration. None of these canaries, however, showed the necrotic liver lesions which seemed to characterise the natural disease.

Canary 3, placed in contact with subcutaneous infection, remained healthy. Canary 6, exposed to oral infection, sickened on the nineteenth day but recovered in three days. These two canaries were at a later date fed with food soaked in virulent broth cultures; this treatment was repeated nine days later. Canary 3 succumbed 13 days after the first feed, and showed on *post-mortem* the typical necrotic foci in the liver, simulating the appearance of the natural disease. Canary 6 remained healthy, but when given 0·2 c.c. *per os* one month later it died in seven days; immunity, if any, was evidently very slight or of very brief duration.

Remarks.

The organism isolated appears to be the cause of the disease. According to the results of canary inoculation, massive infection causes acute disease with fatal termination in a few days, with

only inflammatory lesions to be seen on *post-mortem*. The natural disease is apparently more chronic, and necrotic liver lesions, as produced in Canary 3 by a feeding experiment, are usually found. No conclusions can be drawn regarding the source of natural infection.

Hadley[1] in 1918, describes diseases of canaries previously reported. Pfaff in 1905, Zwick in 1908, and Meissner and Shern in 1908, all described necrotic foci in the liver and isolated organisms which conformed, in those tests employed, to the organism isolated in the present case. Zwick considered his organism a probable member of the hæmorrhagic septicæmia group, but Hadley has shown Pfaff's organism to be outside the group. Gilruth,[2] in 1910, described a very similar condition in canaries, with enlargement of the spleen, but his organism was motile and produced gas.

Other descriptions of canary disease relate to gas-producing organisms, Gram-positive organisms, or Para-B like organisms.

Hadley's[1] description of the biochemistry of Pfaff's culture differs slightly from the reactions here described. Litmus milk remained unchanged, and no acid was formed in adonite. Nevertheless, we conclude that the disease here described is caused by an organism classed by Bergey[3] as *Shigella pfaffi*.

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NOTES ON THE PRACTICE OF BENNETT'S TEST FOR SURRA IN CAMELS COMBINED WITH USE OF NAGANOL.

By T. A. Bryan Cocksedge, m.r.c.v.s.

British Somaliland.

It was not till late in 1926 that I was first able to demonstrate Trypanosomiasis in camels in this country. The first case was demonstrated in a native-owned camel, and later I found a trypanosome in the peripheral blood of a camel of the Somaliland Camel Corps.

Since I arrived in the country early in 1924, I had suspected the disease (*a*) on account of certain clinical symptoms and chronic debility cases in the Somaliland Camel Corps; (*b*) from accounts of disease amongst native-owned camels, under the name of "Gindi," "Dukkan" and "Melig."

The trypanosome demonstrated came morphologically into the category or type of the Evansi, and another was later demonstrated in cattle which I place in the type "Vivax."

It was not until late in 1929, after the articles on Bennett and Knowles' diagnostic test for surra had appeared, that I began systematically to use the test and medicinal treatment with Naganol.

Up to going on leave early in 1931 I had had a certain amount of success and was beginning to work out an organised and systematic routine. The officer who did duty for me during my leave was not very enthusiastic about the Mercuric Chloride test, but did not in any way condemn it.

During 1931 I was lucky enough to visit the Sudan, and Bennett very kindly gave me all the information he could and showed me the technique of the test in detail. On arrival back in Somaliland in June I was thoroughly armed to start a campaign against Surra.

I systematically tested every animal in the Corps, company by company, and was amazed to find 16 per cent. of animals affected. I carried on systematic testing, segregating, and treating any reactors with naganol. The blood of all reactors was carefully searched for trypanosomes, and any native-owned animals in which these were found were treated and kept under observation.

In the early days I had used doses of 10 grams of naganol, but later had slightly reduced this on account of the expense.

Any animal in which the trypanosome could be demonstrated was then used as an observation case, and the dose was gradually reduced gram by gram; blood films were made at the third, sixth ninth, twelfth and every three hours up to 24 hours, and finally at the forty-eighth hour, after intravenous injection of naganol.

The results were interesting and instructive.

It was found that the trypanosomes began to degenerate six hours after injection (and to disappear from the peripheral blood any time after the fifteenth hour), and they could never be demonstrated as late as the forty-eighth hour.

By the beginning of 1932 the dose of naganol had been reduced to 3 grams, and I found this efficient as a cure so far as the camels used by the Somaliland Camel Corps were concerned, *i.e.*, Arabian bred and Somali camels.

Up to date, December, 1933, there was not a single relapse, and I am satisfied that 3 gram doses injected into the jugular vein, in a 10 per cent. solution in sterile water, is an efficient and definite cure.

There was another problem which confronted one. Parts of this Protectorate are very heavily populated with native-owned camels which are very heavily infected with Surra. Two questions arose: (*a*) could the owners be persuaded to pay for injections of naganol? (*b*) had naganol a prophylactic action, and if so for what period was there an immunity?

(*a*) This matter arranged itself, as the report of the cure of "Gindi" very soon spread and its efficacy was well vouched for, because the Somali does not spend good rupees unless he gets good value, and for two years now I have had native assistants doing nothing else but travelling about injecting naganol.

(*b*) Is purely experimental, but the routine carried out is as follows: Every animal of a unit of the Somaliland Camel Corps proceeding into an infected area is injected with naganol 3 grams before leaving headquarters; no unit has remained in an area more than three months so far. On return every animal is tested with Bennett's test and retested after three months; up-to-date there has been no case of infection. Such has been my experience, and I maintain that the giving of the prophylactic dose is justified, and, though Bennett does not definitely state that naganol has a prophylactic action, I think the experiments recorded in his latest articles on the subject do justify my routine treatment.

The conclusions I have come to are that by systematic and routine testing of camels by the Mercuric Chloride test, and prophylactic and curative use of naganol, the dose for the various breeds of camel being worked out with the help of the microscope, Surra as an economic proposition in the working camel can be controlled and for all practical purposes exterminated.

My technique for intravenous injection may be of interest.

Stand on the near side of the animal, an assistant taking a good grip of the camel's upper lip.

Place the left-hand fingers under the neck and over the jugular furrow on the off-side, pressing the thumb well down over the vein on the near side; the vein will in a few seconds stand up well, and an ordinary $1\frac{1}{2}$-in. hypodermic needle with a good bore can now

easily be passed into the vein. When a good flow of blood is got, release the left hand, attach the syringe and slowly inject, and remove syringe and needle in one action.

In conclusion, I wish to express my thanks to Dr. Bennett in particular, and the Sudan Veterinary Services in general, for all the information and help they gave me when I visited that country, and also to point out how beneficial it is for officers in the Public Service to visit other countries and see what the other fellow is doing.

EDITORIAL.

THE DOUBLE INTRADERMAL TEST FOR TUBERCULOSIS.

The article on the double intradermal test for tuberculosis which appears at an earlier part of this number of the Journal cannot fail to intensify the doubts that have been widely felt regarding the value of that method of diagnosis since it was first used in this country in 1925. The facts recorded in it show that in a small dairy herd producing Grade A (Tuberculin-tested) milk the application of the test in accordance with the regulations of the Ministry of Health had disastrous results, since it led to the condemnation of nearly half of the animals on the assumption that they were affected with tuberculosis. And, unfortunately, there is no good ground for supposing that this result was either accidental or highly exceptional in connection with the employment of the test.

It is true that in this particular herd the errors in diagnosis were prejudicial only to the interests of the owners and not to the health of the public, since although they led to the sacrifice of healthy cows they did not sanction the sale of milk from diseased cows. But no one who is well acquainted with the manner in which these tests are being carried out throughout the country can feel any confidence that the designation "tuberculin-tested" means milk exclusively derived from cows that are free from tuberculosis, which is what the public are expected to believe.

The Ministry of Health appears to refer veterinary surgeons who experience difficulty in interpreting the results of the test to the report on *Tuberculin tests in Cattle,* which was published by the Medical Research Council in 1925. That report was the fruit of the researches of a Tuberculin Committee appointed by the Medical Research Council in 1920, to undertake and direct investigations into the methods and results of the use of tuberculin for the diagnosis and treatment of human tuberculosis.

At the time when the Committee was appointed, its seven medical members shared the general opinion that tuberculin tests, and in particular subcutaneous tests, were of proved utility for the diagnosis of tuberculosis in cattle. It was not then contemplated that the subject of tuberculin tests for cattle should be investigated, and no veterinary surgeon or representative of live stock interests was included among the original members. Later five other

members were added to the Committee, one being a veterinary surgeon and the others representatives of live stock interests.

In the early part of the year 1921, representations were made to the Ministry of Health by three stock owners to the effect that "further and better investigation of the value of the familiar tuberculin tests in cattle" was urgently necessary. "It was alleged that the tuberculin tests made in herds producing certified milk frequently gave wholly anomalous results, and that stock owners and the veterinary surgeons who carried out the tests were often unable to state definitely whether tested animals were or were not infected with tuberculosis." The tests generally employed were those depending on the subcutaneous and ophthalmic reactions. It was also asserted that "on the one hand a valuable cow condemned as tuberculous on what appeared to be a positive subcutaneous test might be found, on autopsy, to be free from the disease, while on the other hand an animal which had passed the test triumphantly might, on *post-mortem* examination, prove to be riddled with tuberculosis," and that "a thorough study of the literature and reference to the authorities both in this country and abroad had failed to clear up these conflicting results."

The representations in question were referred by the Ministry of Health to the Medical Research Council, who invited the Tuberculin Committee to consider these problems in cattle with special reference to the hygiene of the milk supply. The Committee, having found that these statements were corroborated by many other stock owners, decided to include the value of tuberculin tests for cattle in their programme.

A survey of the literature on the subject led the Committee to think that the most noteworthy additional method—that is, additional to the subcutaneous and ophthalmic tests—was the application of the intradermal test to cattle. That had been originally employed for ranch cattle in America and "the results obtained when the test was carefully and skilfully made were apparently satisfactory, and quite equal in accuracy to those obtained by the subcutaneous test."

The Committee decided to adopt the method which had been used by Christiensen and Stub in Denmark, namely, the intradermal injection of the tuberculin at a point in a shaved area on the skin of the neck, but modified to the extent that two injections, separated by an interval of some days, were to be made at the same site in the skin.

The Committee were inspired by a desire to find "a trusty and simple test by which the presence of a tuberculous animal in a herd may be speedily detected, this being equally desirable in the

interests of the consumer of milk, of the cattle owner, and of the public health."

Four years after the inception of its investigations the Committee formulated its conclusions, of which the following are the most important:—

" 1. The subcutaneous tuberculin test appears to be a perfectly satisfactory test for the presence of tuberculosis in cattle when carried out under the laboratory conditions of a scientific trial. It is not a satisfactory test when carried out under ordinary farm conditions: the discrepancies then prevailing are often so numerous as to vitiate the general application of the test for practical purposes.

2. Of the different tuberculin tests, the intradermal test as described in this report appears to be superior to the subcutaneous tests, while the ophthalmic test must be regarded as a subsidiary test.

3. The intradermal test is an accurate test for the presence of tuberculosis in cattle, the percentage of error being extremely small. In support of this statement it may be mentioned that in the Committee's experience animals diagnosed as tuberculous by the intradermal test which have not shown tuberculosis on naked eye examination *post-mortem* have been proved to be tuberculous by microscopic examination and inoculation of guinea-pigs."

The majority of the tests carried out by the Committee were made in one herd which had the following history. It had been in existence since 1908, at which time it comprised four animals that had been tested with negative result; and from that time onwards no animal was admitted to it without first passing the tuberculin test, and being retested at the end of three months and thereafter annually or semi-annually. The tests used were the subcutaneous and the ophthalmic. In 1922 the herd comprised 94 cows.

On one occasion (date not stated) tuberculosis had been introduced into the herd through the purchase of some animals that had been tested by a brand of tuberculin that was later found to be unsatisfactory. In consequence, when the herd was next tested (in November, 1921), with Pasteur Institute tuberculin, 14 cows in the herd reacted and were at once removed from the herd. Eight other cows for various reasons were looked upon as " suspicious " cases, but none of these gave the slightest indication of reaction to the tests carried out by the Committee.

One of the striking things in the Report is the omission to call attention to this herd as an example of what can be done with the

aid of the subcutaneous and ophthalmic tests. That, however, sinks into insignificance when compared with the following.

The Report states that the Committee had at the outset made a survey of the literature on the subject, and it contained a list of 31 references to papers by British, American, French, Danish and Swedish authors on tuberculin testing. And it was after a study of these that they formulated the conclusions quoted above, which condemn the subcutaneous test as practically useless under the conditions of ordinary farming. It will be noticed that the Committee's survey included Denmark, but the Committee had a blind eye for the rest of Scandinavia. That was a pity, for years before the Report was issued the following facts had been published throughout the world.

In Sweden, in the year 1908, 457 herds had already been freed from tuberculosis. These herds comprised 18,719 animals. At the commencement of the campaign the herds comprised 16,852 animals, of which 5,092, or 30·2 per cent. were reactors. The average number of animals per herd was a little over 41. The test used was the subcutaneous one.

In Norway, between 1895 and 1913, there had been tested 39,672 herds with 324,422 animals, that is, from about 1,500 to 3,500 herds and from about 12,000 to 27,000 animals each year.

In 1897, of 2,136 herds, tuberculosis was found in 577, or about 27 per cent. and of 24,765 cattle, 2,056 were tuberculous, or about 8·3 per cent. The number of infected herds and animals subsequently decreased each year, until in 1912, of 3,463 herds tested, tuberculosis was found in only 288, equal to 8·3 per cent., and of 20,439 animals, only 829 reacted, or about 4·8 per cent.

Writing with the authority conferred by his great experience in connection with the campaign against the disease in Norway, Malm stated " I consider the subcutaneous test with tuberculin to be as good as infallible when certain precautions are taken."

The precautions referred to were with regard to the source of the tuberculin, the proper dose, and the hours at which the temperature should be taken after the injection of the tuberculin.

One is compelled to believe that these facts were not known to any of the members of the Committee, and it is difficult to find a valid excuse for such an oversight. If they had been known the Committee's conclusions regarding the merits of the subcutaneous test would have been very different.

There is, however, a defect in the double intradermal test apart from the fact that it appears to have a large proportion of errors. That is the difficulty of recording its observed effects in writing—a

defect which makes it especially unsuitable for the cases in which it is at present mainly used, *viz.*, in testing herds with a view to obtaining a licence to sell milk as "tuberculin-tested." In the public interest it is essential that the results of the test of each animal must be submitted to careful scrutiny by some responsible person—a duty which, it is understood, it as present discharged by an officer at the Ministry of Health. He will receive deep sympathy from anyone who will examine the samples of the reports of tests (*see* pp. 46 and 47) on which he must decide whether to accept or reject the opinion of the veterinary surgeon who carried out the tests.

Even the interpretation of the results of subcutaneous tests in the shape of figures showing the animal's temperature at specified periods sometimes presents difficulty, but it is simplicity itself in comparison with the cases in which diagnosis must depend to a large extent on an appreciation of the heat, consistence, form, and sensitivity of the patch of skin and subcutaneous tissue at the place where the tuberculin was injected. And the Tuberculin Committee were firmly of opinion that these things must be taken into consideration, and that the diagnosis must not be based simply on the increased thickness, if any, of the double fold of skin.

The written certificates of the tests which are sent to the Ministry of Health represent the practitioner's individual judgment, and it is idle to pretend that they can be reviewed or challenged like the temperature chart of an animal subjected to the subcutaneous method. And this objection applies with equal force to the employment of the double intradermal test in private practice. The veterinary surgeon who has made the test cannot hand either to the buyer or the seller a certificate that carries on its face anything of the value of a temperature chart.

In consequence of this state of affairs, and because the accuracy of the tests is rarely controlled by *post-mortem* examination, one cannot expect that the Ministry of Health will ever obtain material on which to base a critical judgment regarding the value of the double intradermal test, and the veterinary certificates which the Ministry has accumulated will probably remain waste paper.

The Tuberculin Committee in 1925 thought that the difficulty of finding reliable tuberculin was a serious obstacle in the way of obtaining accurate results with any of the tests, and they appear to have discovered some bad examples. That point, however, never had serious importance in connection with cattle testing in this, or indeed in any, country. It offends commonsense to express such an opinion in view of the results obtained in Sweden, Norway,

the United States and other countries. At least 90 per cent. of the tuberculin used by veterinary surgeons in Great Britain before 1925 was obtained from two sources, and the reliability of either product was never seriously challenged.

It is fairly obvious that the difficulty in connection with tuberculin began with the attempts to standardise and manipulate it in order to reduce the risk of reactions from non-tuberculous animals. The most recent efforts in this direction is to use tuberculin obtained by cultivating the tubercle bacilli on a synthetic medium, one certain effect of which is to diminish the crop of bacilli below what is obtainable by the method used in the manufacture of " old tuberculin " in every part of the world. This attempt to account for positive reactions obtained from healthy animals tested by the double intradermal method is not to be taken seriously, and it is very fortunate that the Ministry of Health chose the tuberculin that was to be used to clear up the obscurity of the results of previous tests described in the article by McEwen and Roberts. In a distant way the results of the final test recall the narrative about Baalam and his ass. The tuberculin did not say what was expected.

The Tuberculin Committee, although they rejected the intradermal test (not to be confounded with the double intradermal), thought that its value " had been well established in this and other countries." But they fell into the curious error of supposing that it originated in the United States and was there employed because it was " the only method practicable for the testing of ranch cattle," and thought that cattle in this country could not well be restrained in a similar manner to that employed on ranches.

The intradermal test did not originate in America, but in France. It was adopted in the United States, and is the method which has yielded such strikingly successful results in dairy and other herds throughout that country.

Enough has been said to show that through lack of knowledge and experience the Tuberculin Committee appointed by the Medical Research Council fell into serious errors, the chief of which was the condemnation of the subcutaneous test in spite of its proved success in the campaign against the disease in Norway and Sweden, and of the intradermal test as employed in the United States with equally satisfying results.

In consequence of these errors, the stockowners and the general public in this country have been misled and progress in reducing the volume of milk containing tubercle bacilli has been delayed.

None of the methods of tuberculin testing is perfect, nor is a

method with any claim to be so called likely to be discovered, but, as we have shown, there are two methods that have stood the test of trial on a huge scale, and no country that can find the means to finance a scheme of eradication need now hesitate from fear that it would fail because of inability to diagnose the disease.

When the object is to stamp out tuberculosis from a herd complete reliance should not be placed on one method of testing. When intelligently employed two different methods can reduce the chance of error nearly to the vanishing point.

It is a significant fact that no country has adopted the test recommended by the Tuberculin Committee, and it is not likely that it would ever have been used in Great Britain if its employment in testing herds to obtain a licence to sell "tuberculin-tested" had not been imposed by the Ministry of Health,

ABSTRACT.

EPIZOOTIC ABORTION AND UNDULANT FEVER IN SWEDEN.[1]

History of Epizoötic abortion in Sweden.—The date of the first appearance of epizoötic abortion in Sweden cannot be determined with certainty, nor is it possible to say where it first appeared, but it was first observed in 1860, in the region of Hälsingborg.

The year 1859 is an important date in the history of abortion in Sweden, because that was the year in which a column headed "Abortion" was first included in the annual reports from veterinary surgeons. At first these reports included only a few cases of the disease, but in the course of time the number increased considerably. In 1859 there were 24 veterinary practitioners occupying an official position (District Veterinary Surgeons). The appointment of County Veterinary Surgeons was not created until 1878; there were then 250 veterinary circumscriptions or districts, and the number of veterinary surgeons had increased to 558 in 1930.

From the small figure of 30 cases of abortion reported in 1859 the number increased without interruption until in 1927 the reported cases numbered 8,208, and the infected herds 1,235. The figures for the next three years were:—

1928, infected herds 1,305, cases 6,649.
1929, infected herds 1,381, cases 7,628.
1930, infected herds 1,764, cases 6,899.

Serological Tests for Diagnosis.—A comparative examination of various antigens showed that it was indifferent whether the antigen was prepared from the human or the bovine type of Brucella. In respect of the method of preparing the antigen the two methods examined (formolised and heated) were found to be of equal value as a whole and in detail. However, the formolised antigens have given a larger number of non-specific reactions than the heated antigens, and from this point of view the heated antigens appeared to merit preference. There were, however, examples of failures in their employment, a positive serum sometimes giving a reaction difficult to read, while the formolised antigens gave a clearly positive result. But in using heated antigen one perhaps runs the risk of missing a slight positive reaction. From this point of view the simultaneous employment of both kinds of antigen would give a certain measure of security.

It is important that the reading of the results should not be practised with the naked eye. Cases have been noted in which rapid reading of certainly positive results were declared negative. This mistake is especially likely to occur in border-line reactions between the specific

[1] From the work by Erik Henricsson, Stockholm, 1931, Isaac Marcus.

and the non-specific. It is therefore better to read with a lens. The rules that have been formulated as a result of careful study are :—

Normal sera may agglutinate formolised antigens with distinct sediment in a dilution of 1 in 20 or more, and without sediment in a dilution of 1 in 80 or more.

With heated antigen the agglutination with sediment is suspected to be positive in a dilution of 1 in 10, while a reaction without sediment ought to attain a titre of 1 in 40 to be considered positive.

It was not found possible to eliminate normal agglutinin by inactivating serum.

Working according to these methods, the presence of latent infections in infected regions seemed to be commoner than one would have thought in general.

There exists between the reactions of agglutination and fixation of complement an absolute parallelism as a whole and in detail, with some isolated exceptions in favour of the agglutination test.

Geographical Distribution and Characters of Undulant fever.— Although undulant fever has been observed in a great number of countries its frequency is very irregular in each of them, and that is especially true for the infections which have a bovine or a porcine order. In Europe there are only three countries in which undulant fever of bovine origin has occurred with real gravity, namely, Denmark, Switzerland and Sweden. In the American Continent the majority of the cases have been observed in the United States. In the other countries of the world only sporadic cases have been reported. The figures for the different countries down to 1927 were : Denmark, 1,800 ; United States, 1,670 ; Switzerland, 474 ; Sweden, 301 ; Germany, 146 ; France, 57 ; Holland, 50 ; England and Wales, 17 ; Scotland, 2 ; White Russia, 11.

The frequency of the disease from bovine origin is insignificant when compared with the population, even in countries where the figures are highest. It has to be remembered, however, that attention has only been drawn to this infection in recent years, and that consequently the figures which are available do not give a correct idea of the distribution or the frequency of the disease. It is probably more widespread and more frequent than the statistics indicate.

The observations recorded in North America on the occurrence of cases of undulant fever imputed to a caprine infection or a bovine origin led to parallel researches in the Scandinavian countries. In 1927 Kristensen discovered the disease in Denmark, and about the middle of the same year a systematic research by serological diagnosis of the infection in man was begun in the National Laboratory for Bacteriology in Stockholm. These researches had only negative results during the first months, and it was only at the end of 1927 and the beginning of 1928 that the first cases of disease in the country were detected.

Immediately after the first cases of undulant fever* were detected in Sweden, the State bacteriological laboratory began to carry out a systematic search for the disease. All samples of blood sent for diagnosis in cases of fever were tested for evidence of infection with Bang's

* Undulant fever throughout the article means the disease in human beings caused by the *Brucella melitensis*.

bacillus (*B. abortus*), and soon afterwards the same action was taken in other laboratories in the country. The result was that from one region after another cases of undulant fever were reported. It was, therefore, clear that the disease was rather widspread, and it was considered necessary (July 1st, 1928) to require medical practitioners to notify the existence of such cases. From that time onwards it has been possible to obtain exact knowledge regarding the frequency and the localisation of the disease in Sweden.

Up to February 1st, 1931, a total of 301 cases of undulant fever had been reported, and from these figures one could reckon with a relative morbidity of 1·66 per 100,000 human beings (Kling). The disease was fatal in only four cases, and the mortality was thus low (1·33 per cent.). As Kling had pointed out, undulant fever in Sweden appears to present certain peculiar characteristics with regard to the susceptibility of the two sexes and of persons of different ages. Thus the statistics showed that in a total of 125 cases of undulant fever 87 were in men and 38 in women. There were no cases in persons under ten years old, and the maximum incidence was between 20 and 40 years. There were comparatively few cases in persons over 50.

According to the same author, in respect of clinical characteristics the disease in principle does not differ from Mediterranean fever, but from the experience of Swedish doctors so far it would appear that undulant fever in Sweden is less severe, the febrile periods being, as a rule, not so numerous and of shorter duration than in the cases caused by the melitococcus. Complications are more seldom and the mortality less. It is recognised, however, that the importance of the disease must not be under-estimated. It often lasts a long time before recovery is complete and the patient is fit for work, and the disease therefore imposes loss on the individual and on the community.

Although the clinical picture of undulant fever, at least when the disease has lasted for some time, is rather typical, reliance was not placed upon that fact alone, and for the purpose of diagnosis thorough examinations were made in the bacteriological laboratories.

For the purpose of diagnosis use was made of hæmo-culture and serological tests (agglutination and complement fixation).

In the course of such investigations an interesting discovery was made by Olin. In 3,000 samples of blood submitted in the State laboratory to the Wassermann test (for syphilis), a number (0·56 per cent.) were positive to the agglutination and complement fixation tests for the abortion bacillus. All these samples of blood came from patients who had not shown any sign of undulant fever. Olin concluded that one had here to do with individuals who had had an occult or latent attack of undulant fever. From the percentage in these cases, Olin believed that the number of such abortive infections in the country would be approximately at least 30,000.

The facts furnish ground for the view that in Sweden undulant fever is much more frequent than is indicated by the number of cases showing manifest symptoms.

It cannot be said that undulant fever in Sweden prevails as an epidemic. On the contrary, the disease has a pronounced endemic occurrence. The cases are diagnosed now and again with almost regular intervals. During the three years 1928, 1929 and 1930, the number of cases reported were respectively 83, 118 and 95; thus, 100

per annum on an average. The figures showed that the greatest number of cases were reported at two seasons, the months in which the largest number were reported being May and October, and it appears that in Sweden abortion in cows shows the same tendency.

From July 1st, when notification was made obligatory, to the end of 1930, the existence of the disease had been confirmed in 21 of the 24 districts. The highest number of cases were reported from Malmöhus, Stockholm and Östergötland, with respectively 50, 44 and 31 cases, out of a total of 265.

The question which the figures raised was—Is undulant fever a contagious disease which spreads from individual to individual, or is the source of infection to be sought elsewhere than in human beings? In many parts of the country there was a certain concentration of cases of the disease, from which one might have inferred that the infection was transmitted from person to person, but so far not more than one case had been reported from the same family.

In the hospitals also there had been no evidence of infection from patient to patient. It was therefore concluded that, although the possibility could not be denied, this method of infection could have only a subordinate importance.

There then arose the question—Must the reservoir of infection be sought in connection with some of the domesticated animals? As is well known the melitococcus disease, that is, Mediterranean fever, is derived from the goat, and it was therefore decided to examine whether there was any evidence to indicate that the undulant fever in Sweden had the same origin. However, a comparative study of the topography of undulant fever and of the presence of goats in Sweden made such an hypothesis improbable. The disease has been most frequently diagnosed in middle and south-west Sweden, but these are the places in which one seldom or never sees goats. *The goats are kept principally in north Sweden, and that is the place where undulant fever is uncommon.*

It was found that Swedish veterinary surgeons have no knowledge of contagious abortion among goats, but experience has shown that in the Mediterranean countries melitensis infection in goats is often manifested by abortion. It was therefore conceivable that Swedish goats might be carriers of a latent infection. The author therefore had 50 blood samples tested from goats in different parts of the country, with the result that the agglutination and complement tests had a negative result in each case. This compelled the conclusion that Swedish goats are free from Melitensis and Abortus infection.

In the southern departments of France undulant fever appears to be, for the most part, connected with infectious abortion in sheep, and the possibility that cases of undulant fever in Switzerland had the same origin had to be considered.

In Sweden, however, sheep breeding takes place throughout the whole country, but it is extensively practised only in one province, namely Götland, and in that province no case of undulant fever has yet been observed. On this ground alone, it was thought very improbable that sheep could be the carriers of undulant fever. Besides, there was no evidence of the existence of an infectious abortion among sheep in Sweden. Finally, the possibility of there being a latent infection of the disease among sheep was contra-indicated by examining 50 samples

of blood from sheep in Götland and finding that they were all negative to the agglutination and complement tests.

A very large proportion of the swine in Sweden are in the regions where the most cases of undulant fever have been observed. The possibility that they were the source of human infection had also to be considered, and that was the more necessary since many of the cases of undulant fever in the United States had been ascribed to a porcine source. It was found, however, that no evidence of infectious abortion among pigs could be found in Sweden, and here again the agglutination and complement tests of samples of blood from pigs had negative results.

It was thus pretty clear that goats, sheep and pigs had no importance in connection with the occurrence of undulant fever in Sweden, and there were no more reasons for thinking that any of the other domesticated animals—horse, dog, cat or fowl, were concerned in human infection.

The matter was very different when one had to consider cattle in this connection. When the first undulant fever cases were discovered contagious abortion was already spread throughout nearly the whole country, but there were certain areas which had not been invaded, or had for a long time been freed from the disease. This circumstance naturally formed a good starting point for the study of the question whether there was any connection between undulant fever and contagious abortion in bovine animals.

In April, 1929, the number of known cases of undulant fever was 125, and information was obtained regarding the places where the patients had been living shortly before the attack.

When this information was examined it was found that none of the cases of undulant fever came from those parts of the country which were considered free from contagious abortion, while on the other hand cases of undulant fever came from the regions badly infected with epizoötic abortion.

During the years since that discovery was made the undulant fever in Sweden has always shown the same topographical peculiarity, in being confined to the places where contagious abortion occurs among the cows. Hence, it may be said regarding undulant fever in Sweden: *No undulant fever where there is no epizoötic abortion in cattle.*

There were thus discovered strong epidemiological and epizoötiological grounds for the conclusion that cattle constitute the source of infection for undulant fever in Sweden. Further important support for this view was provided by the following observation. During the summer, 1928, it happened that one of the assistants in the State Bacteriological Laboratory, in manipulations with a strain of abortion bacilli of bovine origin, accidentally sucked into his mouth a small quantity of bacillary emulsion. Notwithstanding immediate rinsing out and disinfection of the cavity of the mouth, twelve days later there began the symptoms of what proved to be undulant fever. There had thus been produced unintentionally an experimental infection of a human being with a brucella strain of bovine origin.

The question which next arises is—How is this transference of the infective material from cattle to human beings effected?

According to certain observers, in the transmission of the infection from the cow (Kristensen) or pig (Meyer) to human beings contact plays a great part, because most cases of undulant fever had been

observed in the class of persons who are brought into intimate connection with cattle or pigs. According to Kling, however, that does not apply to cases in Sweden. Of 125 patients no less than 101 belong to callings which did not bring them into contact with cattle, while a possibility of infection through contact with these animals could not be excluded in 24 cases (veterinary surgeons, people on the land, or persons working in the cow houses or in connection with milk). A remarkable fact was that almost the whole of the patients had been using unboiled or unpasteurised cow's milk. This naturally was the case also with the individuals whose occupations had brought them into contact with cattle.

It is well known that in cows affected with the disease the bacilli are excreted for a long time with the milk. Owing to the fact that it is impossible to distinguish between the microbes which are respectively the cause of the disease in human beings and in cattle, one must conclude that the milk of cows is one of the media through which the infective agent of undulant fever can reach the human subject.

It thus appears that at least in Sweden contact with infected cattle plays a slighter rôle in the causation of undulant fever than infected milk, and that appears to be borne out by a comparison of the morbidity of the disease in the country as compared with towns. The following table shows this difference.

	Population.	*Number of Cases of Undulant Fever,* 1928-30.	*Morbidity per* 100,000 *Inhabitants.*
Country :	4,162,761	108	2·5
Towns :	1,942,429	120	6·1

Similar conditions appear to exist in Switzerland. The disease is specially prevalent in the large towns, such as Zürich, Bern, Lausanne, and Basle.

For both categories, town and country, of the population, there is one epidemiological factor, namely, infected cows' milk, since (or if) this also in the unpasteurised condition is used to a larger extent in the country than in the towns. On the other hand, contact with diseased animals can be important only for one of the two groups, but how great this importance is naturally cannot be exactly determined. In this connection, however, it ought to be remembered that in Mediterranean fever the milk of goats is regarded as the chief vehicle of infection.

What has been said regarding milk naturally applies to milk products in which the bacteria can remain alive, such as butter and cheese. In Sweden, however, no examinations have been carried out to ascertain to what extent these products are dangerous. On the other hand, there is some information on this point regarding the *Micrococcus melitensis*. It has been shown that the virus of undulant fever can remain alive in fresh cheese for 15 to 44 days, and during that period multiply (Neri). Dubois thought that even the fermenting kinds of cheese might be dangerous, but he was convinced that cheeses of that sort, and especially Roquefort, were devoid of danger in this way. Hauduray and Pacault were of the opinion that the use of fresh cheese involved as great danger as the use of milk.

One has also to reckon with the possibility that the use of the urine of cattle for manurial purposes may cause a contamination of kitchen vegetables with Bang's bacilli. Naturally one must look for possible infection in that way when there is nothing to suggest infection through milk or direct contact with cattle.

In such cases it would also be well to consider whether or not infection had been caused through drinking water. The author has discovered weighty reasons for thinking that cattle can be infected in this way. In his experience it is not unknown in the country for the people and the animals to have a common water supply.

Studies Regarding the Nature of the Swedish Undulant Fever Virus. —Although it must be regarded as proved that cattle form the source of infection for undulant fever in Sweden, it does not follow that the cause of this disease is identical with the bacillus discovered by Bang and Stribolt. Doubt with regard to this identity had already been expressed. It is well known that Bang's bacillus was for a long time regarded as devoid of danger. As the epidemiological observations regarding the occurrence of undulant fever outside its original territory began to accumulate, the suspicion arose that the *Micrococcus melitensis* had found an entrance in new lands and infected the cattle there (Nicolle, Burnet, and Conseil). It is now known that there are several pathogenic organisms which possess the power of causing different morbid conditions and which are very nearly related without being absolutely identical. It therefore appeared necessary to subject the undulant fever virus to a closer analysis.

For a long time after Bruce (1887-88) had discovered the microbe of Malta fever, and Bang and Stribolt eight years later (1895) had discovered the cause of epizoötic abortion, strange to say, one did not think of the possibility that these two microbes might be nearly related. First in 1918 attention was called to the great resemblance between them by Alice Evans in her studies of the bacterial flora of cow's milk. She found, for example, that the antigenic properties of the two microbes nearly corresponded. One then remembered that a micro-organism resembling Bang's bacillus was found by Hutyra, and also by other researchers, in aborted pig fœtuses, and one therefore began to study the relationships of these organisms as found in human beings, goats, cattle and swine. It soon became clear that they were very nearly related, since it was difficult to distinguish them, although Feusier and Meyer (1919) thought they could do so by the absorption method. It was this narrow relationship between these organisms which induced Meyer, Shaw and Fleischner to introduce the name *Brucella* for the group (1922). Later the three different types or variants were named respectively caprine, bovine and porcine.

For the purposes of the diagnosis of undulant fever in the State Bacteriological Laboratory in Stockholm one has generally been content with the serological reactions, but hæmo-culture has also been tried and in a very large percentage of cases given a positive result. In this way, one has come into the possession of a very large number of strains of the undulant fever virus. A good opportunity was thus offered to carry out investigations on newly isolated strains, and to compare them with other known brucella types (melitensis and porcine strains which had been obtained from abroad as well as with strains isolated in Sweden). The study extended to the morphology, cultural and bio-

chemical characters, and pathogenic properties of the different types.

A number of different investigators were engaged in this enquiry, and Kling made a report on the subject at the International Microbiological Congress in Paris, 1930. The following extract is made from that report.

"1. It has not yet been possible to discover distinctive characteristics between the human and bovine strains in respect of morphological, cultural, or biochemical and antigenic properties.

2. The human and bovine strains on the one hand were distinguishable more or less from the porcine and caprine strains with regard to their biochemistry. (Methods of Hardy and of McAlpine-Slanetz.)

3. Cross agglutination reactions do not enable one to differentiate the before-considered types.

4. All our human strains have the power to provoke in the organs of the guinea-pig the same lesions (proliferations, necroses, abscess), although more or less accentuated than the porcine and caprine strains. The bovine strains which we have employed for comparison have been found to be less virulent on the guinea-pig. We do not wish for the moment to pronounce definitely on this character of the *Brucella abortus* of bovine origin since we have not yet had the opportunity to examine a sufficient number of strains."

Undulant Fever in Animals.—The author now turns to the question of the action of the Swedish undulant fever virus on certain of the larger domesticated animals, which, according to the programme of work, was to be studied by the author and Lindström, and which is to be made the subject of a separate publication.

It was naturally first of supreme interest to find out whether the Swedish undulant fever possesses the power to produce morbid changes in the common domesticated animals, especially in those which are known to be susceptible to Bang's infection.

Two series of experiments were carried out; in the first, cows, swine, sheep, goats, mares and bitches, all pregnant, were inoculated intravenously, and in the second animals of the same species, with the exception of the mare, were given infective material by the mouth.

The effects of the intravenous inoculation were as follows:—

Cow	Aborted after 14 days	The virus was found in the fœtus and membranes.
Sow	Negative	Negative.
Ewe	Aborted after 22 days	Fœtus and uterus.
Goat	„ „ 60 „	Membranes.
Mare	Negative	Negative.
Bitch	Aborted after 30 days	Uterus.

It is to be noted that although the sow and the mare did not abort the blood of all six animals was positive to the agglutination and complement tests.

In the second separate series of animals infected by the mouth the results were as follows:—

Cow	Aborted after 92 days	Bacilli found in the membranes and uterus.
Goat	„ „ 30 „	Bacilli found in fœtus and membranes.

In the sow and bitch the outward result of the infection was negative, but the blood of all five animals was positive to the agglutination and complement tests.

It had been found difficult to get sheep at the appropriate stage of pregnancy for the experiments, and the above one on sheep was not concluded at the time of publishing.

Susceptibility of Monkeys.—Three monkeys (*Macacus rhesus*) were three times fed with a suspension in normal saline of a 24 hours culture of undulant fever (10-15-100 millions). All three animals showed a positive agglutination reaction as early as ten days afterwards and the complement test was positive after 45 to 56 days. In two of the monkeys fever set in on the fortieth day and continued for seven days, and in both the bacilli were found in the blood.

There are still certain provinces in Sweden which have so far remained exempt from undulant fever although contagious abortion occurs there among the cattle, and this fact suggested that perhaps the animals there were infected with a special brucella type not pathogenic for the human subject. From one of these provinces abortion material was obtained and a strain of bacilli isolated. With this, experiments were carried out on three monkeys (two *Macacus rhesus* and one *Macasus cynomolgus*). The results were as follows :—

In one the result was negative to agglutination and complement tests and hæmo-culture, but there was an elevation of temperature of an unknown nature after 42 days.

In the second the agglutination test was positive after ten days and the complement test after 42 days. There was fever beginning on the thirty-fifth day and lasting for five days, and blood culture was positive after 25 days.

In the third monkey (*Macacus cynomolgus*) the agglutination test was positive after 14 days and the complement test after 28 days. There was no fever and blood culture failed.

In one of the animals there developed a typical infection, although clinically the attack was slight, with antibodies and bacteria in the blood. In the other two monkeys the blood examination was positive, but that was the only observable effect of the infection, and the third monkey was completely refractory to the bovine strain.

The results of the comparative studies in the State Bacteriological Laboratory regarding morphological, cultural and biochemical properties of the different brucella types as well as their effect on guinea-pigs have been described in what precedes. What was of greatest interest in these results was that the Swedish undulant fever virus in these respects was not distinguishable from the *Brucella abortus bovis*.

It is possible to produce by intravenous inoculation or *per os* specific abortion in the cow, sheep and goat; that had already been done by Bang and other researchers in experiments with the true abortus bacillus. There therefore now remains no distinction between the two pathogenic organisms.

On the other hand, the pig appears to be refractory to the undulant fever virus (no abortion and no bacilli recognisable). It is of interest in this connection that, according to Hayes' experiments, abortion can easily be caused in swine by intravenous inoculation of *Brucella abortus*

bovis, and by means of infection *per os* that researcher was able to recognise the presence of the organisms in the normally born young.

With regard to the question of the virulence for *Macasus rhesus* and *cynomolgus* (by feeding), the human and bovine strains used exhibited no striking difference. Both caused a typical diseased condition in these experimental animals. Judging from the experiences of other researchers with regard to caprine and porcine strains, however, it would appear that their ability to produce fever is comparatively weak.

Relationship between Undulant Fever Virus and the true Bang's Abortion Bacillus.—It has already been shown that in the present state of our knowledge it is impossible by laboratory methods to distinguish between the virus which is the cause of Swedish undulant fever and the abortion bacillus isolated from cattle. The problem of the identity or non-identity of the two organisms can, however, be attacked from another side. What does the study of the epidemiology of the disease say on this question?

Kling had pointed out that in Sweden undulant fever had a tendency to occur in separate centres, and the more we get to know about the disease the more distinctly does this character appear. Already in 1929 one could, in scrutinising the undulant fever maps, detect larger and smaller groups of cases as well as isolated cases. With respect to the fact that large areas of the country were free from undulant fever notwithstanding the occurrence of abortion infection in cattle, the supposition occurred that there must exist a special strain, variant, or race of *Brucella abortus* Bang, which was still limited to certain regions or certain herds, and had become adapted for the human subject.

As this hypothesis appeared to offer a natural explanation of some obscure points in the epidemiology of undulant fever, the author was led to consider the circumstances minutely. For this study he found an appropriate strip-like area in Central Sweden, for here a relatively large number of undulant fever cases has been diagnosed, and at the same time contagious abortion was more or less intensive in the district.

In this region 83 undulant fever cases had been discovered. Of these 16 were isolated, and the other 67 were so near together that they appeared as groups or centres embracing two, three, six, ten to 24 cases. Of these centres there were four, including the two largest, in a district badly infected with epizoötic abortion, whereas the others occurred in a region where the cattle abortion existed only to a moderate extent. The scattered or isolated cases were mostly in regions where infectious abortion shows only a moderate or slight degree of dissemination.

One therefore does not find, as might have been expected, that undulant fever groups are always in regions with intensive abortion infection, but about equally often in districts that are more or less sheltered from the epizoötic. *A parallelism between the intensity of abortion infection in a region and the frequency of undulant fever apparently does not exist.*

In the examined region there were three large towns—Stockholm, Örebro and Västerås, with a population of respectively 500,000, 40,000 and 30,000 inhabitants. In these three towns during the years 1927-29 there were respectively 22, eight, and four cases of undulant fever, corresponding to a relative morbidity of respectively four, 20, and 13 per 100,000 inhabitants. Indeed, Örebro, with the greatest morbidity,

has a milk radius with widespread abortus infection, and the same can be said of Stockholm, with the lowest morbidity of the three towns, where as Västeräs, with a proportionally high morbidity, obtains its milk from one district which can be designated as infected to a medium extent. When one reflects further on the fact that the milk in the three towns in question is delivered in the unpasteurised condition to about the same extent, one can apparently conclude that the occurrence of undulant fever depends not on the quantity of the infective material but on its quality.

Moreover, through a comparison of the frequency of undulant fever in other countries with nearly similar hygienic and demographic conditions, Denmark and Sweden for example, one can obtain a criterion for examining the question of the pathogenicity of different brucella strains for the human subject.

As this table shows, the morbidity in Denmark is about six times as great as in Sweden, and for each 100,000 cows in Denmark there are 23·7 cases of undulant fever. For the same number of cows in Sweden there are only 5·6 human infections. In Denmark undulant fever is

Country.	*Inhabitants.*	*Cases of Undulant Fever during One Year.*	*Morbidity per 100,000 Inhabitants.*	*Number of Cows.*	*Number of Undulant Fever Cases per 100,000 Cows.*
Denmark ...	3,475,000	365	10·5	1,541,000	23·7
Sweden ...	6,081,146	104	1·7	1,865,000	5·6

spread over the whole country, whereas in Sweden it occurs only in certain regions.

Can one regard these figures as indicating that actually there is a human pathogenic variant of the abortion bacillus, and that this is more widely disseminated in Denmark than in Sweden?

This view has been put forward by the Italian researchers (Favilli, Lustig), who think that a human pathogenic variant, capable of causing undulant fever, develops from the common type which is harmless for the human subject. Thomsen, in Denmark, writes: "In this country there appear to be strains of abortion bacilli in cow abortion, and possibly also elsewhere, which are pathogenic for man."

Even if this view is correct one must admit that there are several paradoxical appearances which can with difficulty be reconciled with the hypothesis. In particular, how can one explain the slight morbidity when the organism that is the cause of the disease in man reaches him with cow's milk, which is one of our commonest articles of diet? One must naturally suppose that many human beings must be exposed to infection without being attacked. Most individuals must, therefore, possess immunity, either natural or acquired.

As already stated, there occur in Sweden, in addition to the manifest cases of the disease, a large number of occult infections (Olin), observed in various regions and also at places where no case of undulant fever has been discovered. The wide dissemination of epizoötic abortion therefore compels us to suppose that it is the common abortion bacillus which leads to the development of specific antibodies,

and which can be recognised in a not unimportant percentage of the population. Is it not this acquired immunity which protects most people against the pathogenic variant of Bang's bacillus? In this connection one naturally reflects how nearly related are, in serological respect, the three bacterial types—caprine, bovine and porcine—to each other. This immunisation can, upon the whole, offer a plausible explanation why the morbidity is greater in the towns than in the country districts. In the country, where milk containing abortion bacilli is used very extensively in the uncooked or unpasteurised condition, immunity is acquired in a great many cases. In the towns, on the contrary, a great part of the milk is pasteurised (Stockholm 75 per cent. Göteberg 60 per cent., Malmö 50 per cent.), and only a smaller part of the milk is consumed in its natural condition. This circumstance might quite well have the result that the town inhabitants would not become immunised to the same extent as the country people.

In a study of immunity, Olin made the interesting observation that specific immune bodies are more frequently recognisable in women than in men. This naturally lends itself to an explanation of the different susceptiblity of the two sexes. In the different customs of men and women in Northern lands, with respect to the consumption of milk, it might very well be the cause of the variations in the occurrence of antibodies.

Whether the characteristic insusceptibility of children for undulant fever is due to a natural or an acquired immunity, or both, is a question which cannot yet be answered with certainty.

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